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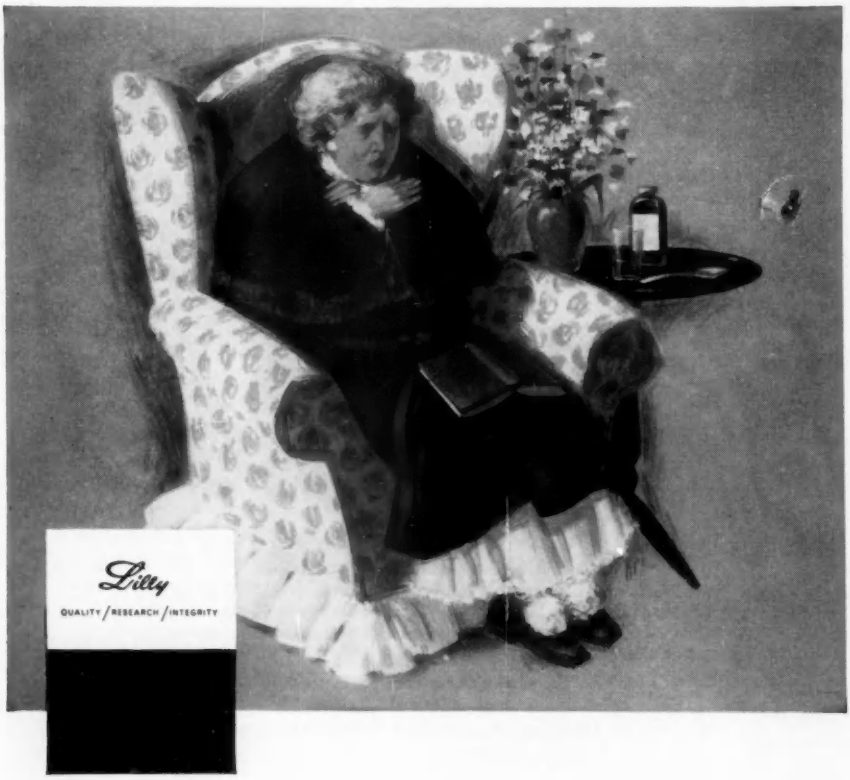

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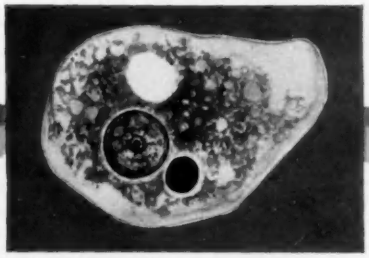

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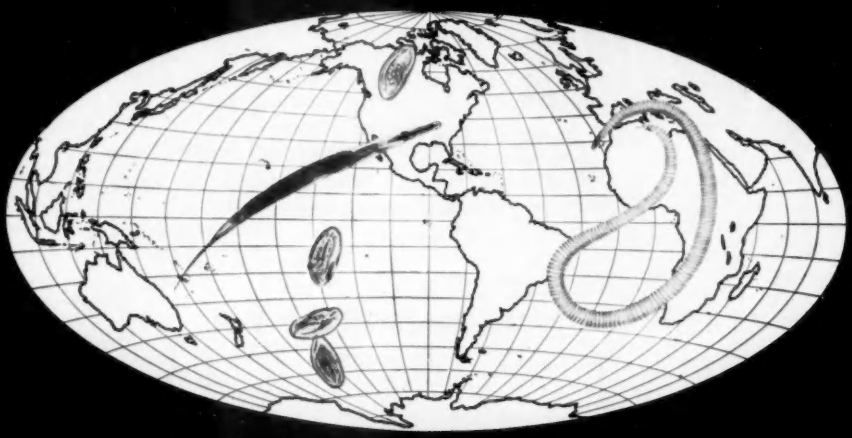

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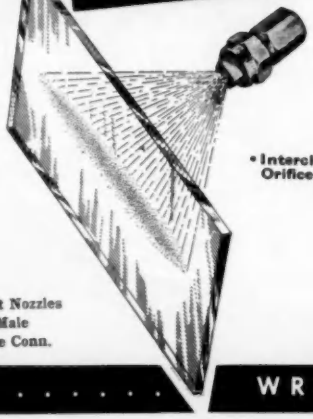

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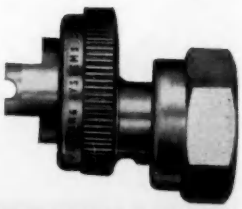

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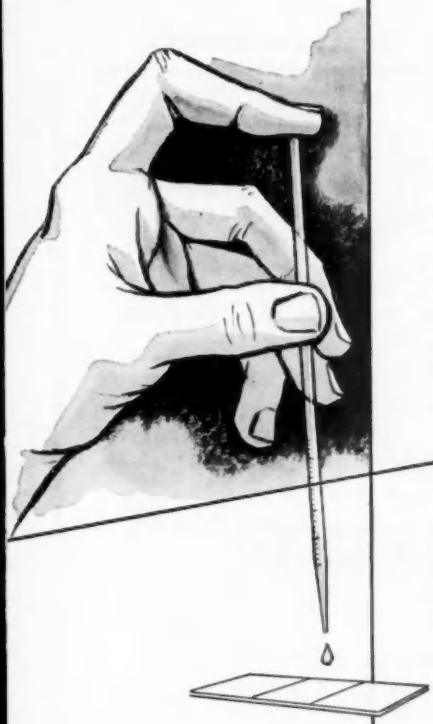

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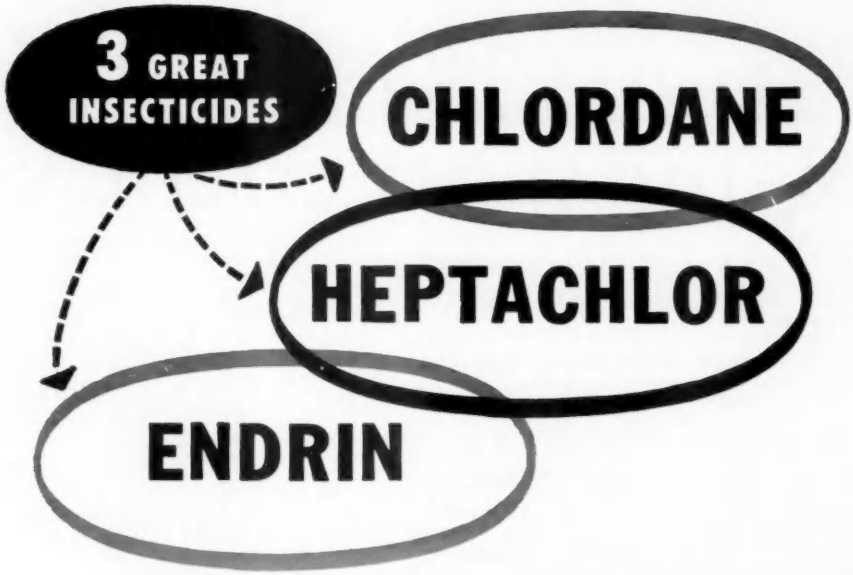

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PRESIDENTIAL ADDRESS[1]

ASA C. CHANDLER

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I want to express my deep appreciation of the honor the members of this Society have done me in making me their president during the past year. I consider it one of the highest honors I could have received. It really is an honor rather than a job, for nearly all of the hard work has been done by our very energetic and efficient secretary-treasurer, Dr. John L. Larsh, Jr., by the conscientious efforts of our committees, and by our council.

With reference to my subject tonight, I doubt if it would be possible in the whole realm of human knowledge to find a more perfect example of intertwining of cause and effect, with the production of a vicious circle, than that presented by malnutrition and infectious disease in the tropics. These are two specters which haunt the great majority of the inhabitants, from conception to the grave, in the so-called tropics. By tropics, for want of a better term, I include all those areas where the standards of living are such as to produce essentially the same poor health conditions. Before going into detail about the interrelations of infection and nutrition, let us first look briefly at the status of each in the tropics today. The backwardness of many tropical peoples, as Loughlin and Mullin (1955) suggested, is the result of injury by these two specters, rather than of any climatic effects or innate inferiority. These peoples' situation is reminiscent of that of the "poor white trash" of the South in the days when hookworm, malaria and pellagra were rife.

The ubiquity of exposure to infectious disease in the tropics needs little said about it to this audience, but it can not be fully appreciated by anyone who has not lived in those areas. Within his first few years a child in the tropics is exposed to practically all the diseases to which children in our own country are subject, plus a considerable number of additional ones including, in one place or another, malaria, a large variety of arthropod-borne viruses, smallpox, cholera, plague, yaws, relapsing fever, trypanosomiasis, leishmaniasis, schistosomiasis, hookworm, filariasis, and onchocerciasis, to mention just a few. Tuberculosis isn't usually thought of as a tropical disease, but today its highest incidence is in tropical and subtropical countries, especially where industrialization is going on.

The great prevalence of infectious disease is easily understandable when it is realized that three-fourths of the world's population, and all but a negligible number in the tropics, drink impure water, dispose of human excreta recklessly, prepare milk and food without regard to the most primitive sanitary precautions,

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are constantly exposed to mosquitoes, flies and ectoparasites, share their homes with rats, and are inadequately nourished.

Let us now look at the nutritional side of the picture. After such a dinner as we had tonight in which all of us could, and no doubt most of us did, overstuff ourselves, and to a largely diet-conscious audience it must come as a shock to realize that well over half the people on the Earth go to bed hungry nearly every night. Their calorie intake is often one fourth to one third less than the optimum. But common as undernutrition is, malnutrition is still commoner. While lack of vitamins, one or several, has been particularly stressed in earlier work on malnutrition, and everyone is familiar with such vitamin-deficiency diseases as beriberi, pellagra, scurvy, rickets, etc., it is only in rather recent years that the most widespread and serious deficiency in the world today—that of protein—has been given the prominence it deserves. This protein deficiency results not only from inadequate total amounts of protein, but also from poor quality or poor digestibility of the meager supply that is available. The diet in all countries of low economic and educational status consists primarily, sometimes almost exclusively, of cereals or tubers, and usually very largely of a single one—most often rice, corn, millet, legumes or cassava. Without exception these diets are bulky, deficient in usable protein, relatively excessive in carbohydrate, inadequate in one or more vitamins, and frequently low in calories. Less than one-tenth of the world's population have adequate amounts of animal protein, and it takes surprising amounts of vegetable diets to compensate for the resulting deficiencies in such amino acids as tryptophane, lysine and methionine. Often the required amounts are not available, or digestive tracts loaded with *Ascaris* or *Giardia*, are unable to hold or tolerate them.

Unfortunately nutritional deficiencies are most pronounced when most harmful, i.e., in children between the ages of 1 and 5. This is partly because of the way the children are fed, and partly because of the pitifully inadequate feeding of pregnant and lactating mothers. Jeliffe (1955) called attention to the two great transitions in feeding methods to which man, like other mammals, is subject. The first is the transition from placenta to breast feeding at birth, the second the transition from breast feeding to other foods at weaning. Among animals and most human beings the first transition is automatic and beautifully cared for by Mother Nature. Only in the most advanced areas of civilization has man interfered with Nature's method by substituting artificial feeding, a practice only made possible by modern technical developments. A little girl for the first time seeing her baby sister being nursed by her mother, and having Nature's method of feeding babies explained to her, exclaimed, "But, Mother, is it pasteurized?" In the tropics a child seeing a baby being fed from a bottle would only wonder how the milk got from the mother to the bottle.

Not only is the amount of animal milk available for infant feeding negligible in most parts of the tropics, but even when occasionally obtainable it has a very poor reputation, probably because of the gastro-intestinal disturbances resulting from inevitable contamination and heavy bacterial growths in the absence of good transportation, refrigeration or clean containers. On the other hand,

mother's milk is surprisingly good even when from grossly underfed, overworked, heavily parasitized women who have no good dietary supplements to help them and may even be deprived of some of the good features of their ordinary diet by taboos. In Nigeria, however, they used to have "fattening houses" to turn out brides properly equipped for pregnancy, and some of the well-to-do even returned to them for a time after having babies—quite a contrast to our "slenderizing" of prospective brides. Some of our ideas on nutrition no doubt seem just as absurd to natives of the tropics as theirs do to us. An absurdity has been defined as a statement or belief manifestly inconsistant with one's own opinion.

Whereas lactating mothers often suffer severely, the babies, during their first six months or so, are stuffed with good food—quite likely for the only time in their entire lives. In most areas in the tropics breast feeding commonly continues for from 18 months to two or even three years, in fact until interrupted by the birth of a new baby. But after about six months the amount and quality of the mother's milk seldom keep pace with the increasing demands, and some supplementary food is needed. A baby is given scraps of the bulky, high-carbohydrate adult diet, which may include such things as fried bean cakes, black bread, curry or tortillas. If special food is prepared it consists of thin cereal gruels, sugar water, banana paste or other low-calorie, low-protein foods. Often a hungry child, in its second year of nursing, can be seen gnawing on cucumbers, bones, chicken feet or grasshoppers.

Weaning, the second great transition in feeding methods, is a precarious process for human babies, whose self-reliance as well as digestive functions develop more slowly than in most animals. It is only the comparatively recent innovation of the use of animal milk and purified vitamins, still limited to the most highly developed countries of the world, that has made weaning feasible after a few months, or even complete abolition of breast feeding. In most tropical countries some special practices, and many taboos, have been developed to tide the child over the immediate postweaning period, but many of the practices are bad, and the taboos are worse (Jeliffe, 1955). The poor nutrition prevalent at this stage of life is intensified by the impairment of vitality resulting from the inadequacy of the mother's milk during the preweaning period. Exposure to infections borne by flies, fingers and water is increased. At this time, too, a baby is no longer fortified by immunities acquired prenatally, but must build his own antibodies and phagocytes. As a result of the malnutrition itself, or of irritating foods, or of malaria, or of intestinal parasites, or of efforts to remove the parasites, diarrhea inevitably develops, and that of course adds to the nutritional deficiencies. The diarrhea is usually treated by still further restricted diets, limited to a little sugar or cornstarch in water, and minus any tidbits of animal foods that might previously have been present. To this combination of adverse conditions must be added at least three more—loss of appetite, restricting intake even of food that is available; injury to the pancreas, reducing enzymes needed for digestion; and impaired use of such food as is digested and absorbed, because a liver afflicted by fatty degeneration and fibrosis is unable to perform the necessary metabolic functions.

To this unfortunate condition of infantile malnutrition, in which protein deficiency plays the major role, has been given the exotic name "kwashiorkor," an African term meaning disease of a displaced child, i.e., a child who has been weaned to make way for another. Kwashiorkor is a condition that is now known to occur in nearly all parts of the world, the incidence and intensity depending on economic status, and amount and types of food grown (Trowell *et al.*, 1954). Another factor, however, is the abysmal poverty of knowledge concerning nutrition which, as C. D. Williams (1954) pointed out, is sometimes a more potent factor in malnutrition than is economic poverty.

Although it is between the ages of 1 and 5 that the effects of malnutrition are usually most severe, they by no means end there. Not only do dietary deficiencies continue to haunt most tropical natives throughout life, but the damage done in the early years, e.g., to the intestine, liver, pancreas, and pituitary and adrenal glands, may permanently affect the ability of these organs to do their full part in the regulation of absorption, utilization and excretion of the nutrients that are available.

Well, so much for the status of our two interacting factors in the tropics—on the one hand, practically universal exposure to all kinds of infectious agents, the damage they do being therefore determined almost entirely by natural or acquired ability to resist them; on the other hand, almost universal malnutrition, most severe at the most critical age of growth and development. How then do these two factors interact on each other?

As Dean Smith (1955) remarked, the mutually adjuvant effects of malnutrition and infection seem so eminently reasonable and logical, and their coexistence so common, that detailed evidence for their interaction has not often been critically evaluated. An interesting commentary on this is Platt's (1953) observation that in a hyperendemic area in Gambia the reduction of malaria in one village and improved nutrition in another had strikingly similar effects in improving health.

Let us first consider how nutritional inadequacies influence the incidence and course of infectious disease. In a recent review of this question (Chandler, 1953a) I grouped these influences into two categories—A, effects on what we may call the in vivo culture conditions for the parasites, and B, the effects on the defense mechanisms of the host; but even these are inextricably interrelated. In the enthusiasm for studying the effects of a particular ingredient of the diet, the interrelations of any one essential element with others are often lost sight of. Halpern (1955) pointed out that for the most efficient utilization of any one dietary substance, all metabolically related substances must be present simultaneously. In one way or another, and often in several, lack or paucity of a single essential substance in the diet may affect the entire metabolism of the body of the host, and no doubt to a considerable degree that of parasites also. Some interesting examples of the inter-relations of various dietary components are listed by Platt (1956a). These inter-relations undoubtedly account for the remarkably similar effects on parasites described by various investigators as resulting from restriction of proteins, restriction of various individual vitamins, generally poor diet, or undernutrition.

Nutrition of parasites, like that of the host, is a very complicated matter, and its complexity is greatly increased by relative dependence of host and parasite on particular nutritional factors, and by competition for them. Intracellular viruses and rickettsias thrive as the host thrives—they suffer along with the host cells from any nutritional deficiencies. This apparently applies to some extracellular organisms as well. Kühnau (1955) said there was ample evidence, during and after World War II, that extreme protein starvation decreased the incidence and mortality of many epidemic diseases. He assumed that protein intake influences the course of infections not only by enhancing defense mechanisms of the host, but also by providing the invading organisms with materials for growth and for synthesis of toxic substances.

There is no generalization possible concerning the effect of vitamin deficiencies on the course of parasitic infections. Deficiency or increase of a particular vitamin in a particular host-parasite combination may favor either one or the other, or may favor or injure both. Time does not permit going far into this matter but I might mention lack of PABA, as in milk, injuring some species of malaria parasites but not the host; biotin deficiency adversely affecting host more than parasite, and riboflavin deficiency doing the opposite. An interesting instance of adverse effects of certain nutritional deficiencies on both host and parasite was recently discovered by DeWitt (1956). He showed that certain nutritional deficiencies in schistosome-infected mice allowed more worms to develop by lowering the resistance of the host, but at the same time prevented the normal growth and development of the parasites. Similarly Rama Rao and Sirsi (1956) showed that depletion of thiamin in chickens with *Plasmodium gallinaceum* infections retarded multiplication of the parasites, but also hastened death of the host by lowering resistance. Low doses of thiamin benefit the host but not the parasites, so life is prolonged; apparently the host gets access to the thiamin before it becomes available to the parasites, so raises host resistance without stimulating multiplication of the parasites. High doses of thiamin, on the other hand, enable the parasites to multiply so rapidly that they kill the host more quickly in spite of its improved capacity for resistance.

It must be remembered, of course, that particular nutritional factors may have quite different effects not only on different parasites but also, sometimes, on different hosts. Although an exclusive milk diet interferes with development of malaria parasites in both adult and baby rats and monkeys (Maegraith *et al.*, 1952; Hawking, 1954), McGregor *et al.* (1956) failed to demonstrate such action in breast-fed infants in Gambia. A man who had done considerable work on the effects of nicotine on mice, when asked about his conclusions, said he was convinced that mice should not smoke. Probably Dr. Larsh, who worked on some effects of alcohol on mice feels the same way about their drinking cocktails.

The most important effects of poor nutrition on infectious disease are due to interference with repair of injured or destroyed blood or tissues, or with natural or acquired resistance. Repair of damaged tissues, like growth, increases the demands for practically all nutritional elements. Blood loss, particularly in hookworm infections, requires large increases in both protein and iron intake to compensate for it. Janz *et al.* (1956) observed that in cases of hookworm infection

normal serum-iron could not be maintained even on a diet containing 37.5 mg. of iron per day, and postulated that the infection somehow leads to defective absorption of iron, or abnormal fixation of it in the tissues. Pereira Nuñez (1956), on the basis of this postulation of interference with absorption, questioned the value of enriching the food of hookworm-infected people with iron, as advocated by Cruz and de Mello (1945) and others. However, when it is considered that at best not over half of the dietary iron is absorbed (say 20 mg. in the diet of Janz *et al.*, 1956) and that on the basis of Wells' (1931) calculations on the amount of blood lost per day per worm (0.84 cc. of blood containing about 50 mg. iron per 100 cc.), it would take less than 50 hookworms to cause a daily loss of 20 mg. of iron, and that when there is active demand for new hemoglobin serum-iron is low, no interference with absorption need be postulated.

The skin and mucous membranes constitute the first lines of defense against invading organisms. In poorly nourished persons, in whom these membranes are adversely affected, there is, therefore, increased susceptibility to infections that involve primarily the skin, alimentary canal and respiratory system (Keys *et al.*, 1950). The association of ulcers of skin, mouth and genitals with poor nutrition in the tropics is very well known. Frye (1955) assembled numerous references relating to the effect of malnutrition on the invasiveness of amebae, although there is very little effect on incidence of amebae in the lumen of the gut. Elsdon-Dew (1954) observed that there was little difference in the incidence of amebic infection in rural and urban Africans in Natal, but every difference in the incidence of acute amebic dysentery in the more poorly nourished urban residents.

Let us now consider the effect of malnutrition on the subsequent course of infections after their establishment. Non-specific responses, such as inflammatory reactions, hypertrophy, and degradation of toxins come into play, but the principal factor concerned, and one which also influences the others, is antibody production. Antibodies are at the basis of allergic reactions; they tremendously increase the effectiveness of phagocytosis; they participate in the immobilization and death of micro-organisms; and perhaps most important of all, in some cases, at least, they interfere with growth, development and reproduction of parasites by interference with their nutrition (Chandler, 1953b). For example, the striking effects of diet on the course of hookworm infection in dogs, demonstrated by Foster and Cort (1932, 1935) and others, is probably due largely to effects on capacity for antibody production. Amino acids are necessary for building antibodies (Cannon, 1948) and vitamins are involved in their synthesis (Axelrod and Pruzansky, 1955), so antibody formation, if possible at all, is a severe drain on an already depleted person.

Protein deficiency also affects the course of infections by depressing leucocyte production. Since one of the most important modes of action of antibodies is to stimulate phagocytosis, interference with phagocyte production may almost completely nullify the effectiveness of such antibodies as are present (Doan, 1946; Schmidt and Squires, 1951).

It is probable that interference with allergic reaction due to failure of antibody production might also be a factor of importance in some diseases, sometimes good,

sometimes bad. When starvation was rife in Warsaw during World War II rheumatic fever practically disappeared. On the other hand tuberculosis runs a more rapid uninhibited course. In helminthic infections there might be failure of the phenomenon of self cure, i.e., expulsion of worms resulting from allergic reactions of the intestinal mucosa to newly-arriving worms.

Let us now turn to the opposite side of the picture—the effects of infectious diseases on malnutrition. Of course, one way in which parasites intensify malnutrition is by competing with the host for food where the bulk of the parasites is large as in *Ascaris* and some tapeworm infections, or by competing for particular constituents of the diet. In our laboratory (Chandler, 1943) we have shown that tapeworms are able to abstract all the proteins and vitamins they require from the host's own stores, and thrive even when these are entirely absent from the diet, so tapeworms may be able to convert moderate vitamin and protein deficiencies into severe ones. The ability of the fish tapeworm, *Dibothriocephalus latus*, because of its avidity for vitamin B_{12}, to precipitate pernicious anemia in people who are on the borderline of it (von Bonnsdorff, 1956), is a striking example of what may happen. In complete famine, however, tapeworms, because of their dependence on dietary carbohydrates, suffer first, and soon leave the body. Platt (1956a) calls attention to the need for larger doses of vitamin B_1 in treating beri-beri when this disease is complicated by fever. Half of a series of patients with sub-acute beri-beri first noticed their symptoms after a non-specific infection.

Infections also aggravate malnutrition by causing fever, which increases the metabolic rate, and this increases the demand for the food which is already in short supply. Only when the dietary condition gets so bad that the normal febrile response to infection is no longer possible does fever cease to be a factor.

Another effect of infections is to cause anemia. This is most conspicuous in malaria due to the breakdown of hemoglobin, and in hookworm infection, due to the constant sucking of blood and spilling of it into the intestine, but other infections cause extensive anemia in more subtle ways (Platt and Wadsworth, 1956). Infections thus intensify the anemia that a low protein diet produces when there is important competition for the available protein by growth, pregnancy or lactation (Hallgren, 1954). The added loss of hemoglobin in malaria infections and of both hemoglobin and plasma proteins in hookworm infections, may be critical.

One of the most dangerous effects of infections, especially intestinal ones, is diarrhea, often aggravated by vomiting. Dietary deficiencies by themselves can add to their own magnitude by inducing diarrhea, but there is no reason to doubt that intestinal infections are important in this connection. Kwashiorkor is frequently ushered in by a bout of diarrhea precipitated by intestinal parasites or by efforts to get rid of them. De Silva (personal communication, 1956) says that in Ceylon most of the cases begin their illness in this way. I have seen severe malnutrition induced by diarrhea and anorexia of infectious origin even in persons who had been enjoying a perfectly adequate diet. I think many nutritionists minimize the contribution of intestinal parasites to malnutrition because these have about as great incidence in well-nourished as in malnourished individuals,

but there are seldom any data on the degree of infection, or on the injury produced by the parasites. Loss of appetite, an accompaniment of many infections, is undoubtedly an important contribution to malnutrition. This effect, in addition to fever and destruction of hemoglobin, makes malaria one of the more important accomplices of malnutrition in the tropics.

Since metabolic functions of the liver are severely damaged by protein deficiency because of the extreme fatty degeneration which develops in it, it is inevitable that any additional injurious effects on the liver would aggravate the trouble. Such injury is produced by a variety of tropical diseases, including malaria, kala-azar, leptospirosis, yellow fever, virus hepatitis, and schistosomiasis. Maegraith (1948) called particular attention to the effect of malaria in depressing liver functions, and Mousa *et al.* (1955) to the effects of schistosomiasis and its treatment. Infections also intensify malnutrition by impairing metabolic functions in various other endocrine organs, thus interfering with utilization of nutrients that are absorbed (Erschoff, 1952).

Malnutrition aggravates itself by injuring the pancreas (Gillman and Gillman, 1951) and inhibiting output of pancreatic digestive enzymes (Thompson and Trowell, 1952). This effect, too, may be induced by infectious diseases, perhaps more commonly than is realized. Veghelyi (1949) found that scarlet fever, for instance, causes marked changes in the output of one or more pancreatic enzymes.

Any one kind of parasite may influence nutrition in a variety of ways. Two examples, the malaria parasites and *Ascaris*, will suffice. Platt (1956) summarized the possible ways in which malaria might effect malnutrition under two heads: (a) reduction of effective protein supplies, under which he listed anorexia; vomiting and diarrhea; impaired anabolism—due, for example, to liver damage, proteinuria, and abnormal distribution of protein between vascular and other body compartments; and (b) increased protein requirements—due to feeding of the parasite, especially with hemoglobin; excessive protein catabolism; synthesis of the materials of immunity—i.e., antibodies and phagocytes; and replacement or repair of damaged tissues.

Ascaris infection ranks close to malaria as an adjuvant to malnutrition. In many parts of the world it is almost universally present in children and frequently in adults too, and often in surprising numbers—up to hundreds in children and 1,000 or more in adults. De Silva *et al.* (1953) considered that in Ceylon it caused the highest fatality rate of any intestinal parasite, hookworm coming second. *Ascaris* causes ill effects by its migration through the lungs, intestinal blockage, probing into bile and pancreatic ducts or the trachea, allergic symptoms, and neurotoxic effects, but its greatest importance lies in its adverse effects on nutrition. These include loss of appetite, abstraction of food from the host, damage to the intestinal wall, production of anti-enzymes which may neutralize the host's already depleted digestive enzymes, toxic action on smooth muscle which results in colic, diarrhea and vomiting, and in "intestinal hurry" that decreases food absorption (Jelliffe, 1953). Even when the worm burden is low, the loss of nitrogen in the feces is reduced after expulsion of the worms, and the absorption of amino acids is markedly improved (Venkatachalam and Patwardhan, (1953).

Spindler (1947) showed a definite retardation of growth in piglets infected with only a few *Ascaris*.

Sometimes attempts at treatment of infections may aggravate malnutrition even more than the infections themselves. Many commonly used drugs have low therapeutic ratios even in persons not enfeebled by malnutrition, and may cause loss of appetite, vomiting or diarrhea, anemia, interference with vitamin synthesis in the intestine, and injury to the liver. Autret and Behar (1954) stated that children in Central America admitted to the hospital with kwashiorkor had often recently been given an anthelmintic. Fortunately some of the newer drugs, such as piperazine, hexylresorcinol, and chloroquine are less harmful than many older ones, and addition of vitamins to orally administered antibiotics has been very helpful.

It appears from what has been said that infectious disease and malnutrition are so inextricably interwoven with each other that any attempt to deal with them separately, or separately to appraise their effects on the welfare of people in the tropics is as futile as trying to separate the effects of heredity and environment. A bacteriologist or parasitologist is likely to consider infectious disease as the primary factor whereas to a nutritionist diet is basic. The commonly observed fact that either factor may and does operate without the other, gives strong support for either view-point. Also, especially in infants, malnutrition may be so severe that elimination of parasites, few in numbers and partly suppressed by inherited immunity, may have no obvious effect (Masseyeff, 1956).

In the treatment and prevention of these two interlocking scourges of underdeveloped countries, it seems to me that malnutrition deserves the first consideration, since it is basic to natural or acquired resistance to infectious disease. Even today, with all our advances in therapeutics, specific drug treatment of human diseases is with some exceptions of less importance than natural or acquired resistance, which usually prevents serious consequences when nature is allowed a free hand in a healthy body. Chemotherapy should be considered an adjuvant to natural or acquired resistance, not a substitute for it. In addition, as mentioned earlier, many specific treatments are poorly tolerated by grossly undernourished bodies. How, then, is improvement in the nutritional status to be brought about?

Of prime importance is education. As C. D. Williams (1954) remarked, medical and public health workers have been less effective in altering ideas and attitudes of unsophisticated people than have political, commercial, or religious enthusiasts. Habits that are merely the result of ignorance and carelessness are dignified as "social and cultural customs," and little attempt is made to change them. Much more has been done to eradicate malaria, yaws and tuberculosis than to eradicate ignorance. Residual sprays, drug injections, vaccinations and free skim milk have done great things, but none have involved important changes in age-old ways of life. They are like putting pans under leaking pipes instead of mending the pipes. As Williams said, food distribution and drug injections take the eye of political administrators because of their short-term, spectacular effects, more than do long-range programs of education and development.

Education could not only bring about production of more and better foodstuffs,

and increased utilization of nutritionally valuable foods now locally available but unused, but could also greatly enhance the benefit from the foods that *are* used, by improved methods of handling and preparation. Education is also of prime importance in care of mothers and babies and in the prevention or control of infectious disease and parasites, along with the methods of vector control, therapeutics, and vaccinations mentioned above.

These long-range programs are not easy. They require understanding and skill in their development, for they involve fundamental changes in the habits and ways of thinking of people completely unfamiliar with modern ideas and methods. Platt (1954) tells of Gambian natives who tied up a motorcar to keep it from getting loose and causing damage, and of an African who when given a wheelbarrow, loaded it and carted it off on his head. The naïveté of unsophisticated people with respect to food handling is well illustrated by an incident that happened when I was living in India. My wife, who should have known better, went out into the kitchen just before a dinner to tell the cook to make a minor change in the menu. She found him straining the soup through one of the sahib's socks and remonstrated furiously. When the cook could finally get in a word he said, "But, Memsahib, it wasn't a *clean* sock!" Moreover, these long-range changes in agriculture, nutrition and public health cost money and are possible, in the underdeveloped countries where they are needed, only with outside help from the United Nations or from the more prosperous countries, particularly our own. It is a matter of raising the standards of living over vast areas, and it will take much money, much effort, and much time. Why should we be concerned with it?

First of all, in the words of President Eisenhower: "There can be no enduring peace for any nation while other nations suffer privation, oppression and a sense of injustice and despair. In our modern world it is madness to suppose that there could be an island of tranquility and prosperity in a sea of wretchedness and frustration." As I have remarked elsewhere (Chandler, 1956), aside from any moral responsibility, we cannot, on the basis of the economic welfare of our own country, ignore the welfare, particularly the health, of the underprivileged countries, on which we are dependent for surprisingly large amounts of raw materials, a dependence which is steadily increasing. The availability of these materials, and their cost, is inevitably influenced by the health of the people who produce them. In addition, as R. R. Williams (1956) pointed out, the people in whose hands "now reside modern scientific knowledge and technology are outnumbered many fold by the underprivileged people who need our help." If *we* do not aid them, communism will surely attempt to do so, and eventually use these people to destroy us.

To help these people we must help them to help themselves. A very important function of our foreign aid program should, I think, be directed towards improvement of these people's nutrition since in that way we strike most directly at their health, including liberation from infectious diseases, and at their ultimate economic welfare. Since a starving man has to have food before he can produce food, the first necessity, as I see it, but only as a stopgap, is to supply additional food, particularly for vulnerable groups, such as mothers and newly weaned children.

In this country we have an excess of food, providing headaches for farmers, worry for government in handling of surpluses, and unwelcome weight gains for those of us who are trying to stay on diets. A very wise and helpful step in the right direction was made by our government when it passed Public Law 480, which provided a means whereby surpluses may be sold to needy countries for payment in their own currencies, or for barter, or supplied to them on a grant basis to meet famine or other urgent relief requirements. Most of the currency realized from sale of agricultural commodities is used for financing economic developments in the purchasing countries on a loan basis. By the end of 1955 over $2 billion worth of commodities that were a burden to us were provided to needy countries.

The second step, and ultimately much more important, is to help these underprivileged countries to develop their own resources. In this, the United Nations and our own government have made considerable headway. The World Health Organization, the Food and Agriculture Organization, the Institute of Nutrition for Central America and Panama, as well as Point Four of our own government have contributed extensively in studying the needs of the countries, in instituting educational measures, in providing technical assistance in such diverse projects as improving breeds of chickens, developing more nutritious corn or beans, improving the nutrient value of tortillas, devising methods of utilizing soybeans, and in countless other ways. Of course the countries themselves must cooperate by importing tractors and fertilizer instead of cadillacs and canned foods. Industrialization without developing adequate food resources is not the cure-all for under-developed countries that it is sometimes thought to be.

Even greatly improved agriculture does not supply a full solution. It may provide enough vegetable food to prevent starvation, but it will not provide the quality of food that is necessary for much joy of living. There will still be widespread shortages of certain amino acids and vitamins. Only animal protein can adequately supply the amino acids. In some countries increase in livestock production is possible. If trypanosomiasis can be controlled, vast areas in Africa may become a home for contented cows. But in the densely populated areas of the world it is impossible, since it takes seven times as much land to feed a family on meat and milk as it does on rice or corn. R. R. Williams (1956) questioned whether agriculture alone could do the job; chemists may have to come to the rescue by economically producing synthetic supplements—vitamins today, possibly some day the scarce amino acids. Vitamin deficiency diseases have been largely eliminated from our own country by enrichment of flour, and this now is being done in the Philippines by enrichment of rice and in Jugoslavia by enrichment of corn meal. Williams says that all major deficiency diseases of the world except kwashiorkor could be eradicated by vitamin fortification of foods at a cost of about 25 cents per person per year. When it is remembered that with increasing demand thiamin dropped from $10.00 a gram to 6 cents, the possibility exists that cheap amino acid supplements may also become obtainable, although the matter is complicated by the fact that all the amino acids essential for protein synthesis must be absorbed simultaneously and are not so when pure amino

acids are mixed with poorly digestible proteins like zein (Melnik *et al.*, 1946; Seddik, 1950). If use is to be made of dietary supplements in dollar-short countries, it will somehow have to be incorporated in our foreign aid program. Possibly some of the proceeds from sale of surplus goods could be diverted to development of chemical plants for production of these supplements.

In the controversy over foreign aid, many people have taken an antagonistic attitude for various reasons—you can't buy friends; lending money makes enemies; the aid provided is misspent; why be concerned about Africans and Indians when there are people in our own town who need help, etc., etc. There is no doubt that any program, however valuable, can be abused. We can't cut out our military expenditures, or our civic development expenditures, because some of the money is misspent or poorly distributed, or might be useful elsewhere, and this certainly applies to our efforts to improve the living conditions of our less fortunate fellow beings in underdeveloped countries. I believe that the members of this society, who would not be members of it if they did not have a fundamental interest in tropical peoples, should use every possible opportunity to stimulate public interest in improvement of the health and thereby in the economic conditions in underdeveloped countries, and thus further the basic interests of this society—tropical medicine and hygiene.

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INTRODUCTION OF LEON HERBERT SCHMIDT AS THE CHARLES FRANKLIN CRAIG LECTURER[1]

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Mr. President, Ladies, and Gentlemen:

It is a signal honor for me to present this year's Charles Franklin Craig Lecturer. I have known Dr. Schmidt for some 16 years; we worked together during the World War II malaria program and afterwards. During this period, my respect and admiration for him increased continually. During the wartime program, he accumulated and forwarded to other workers data on the basic pharmacology of many potential antimalarials, and these data formed the basis for the safe use of the drugs in human trials. Reports of the trials were frequently published with merely an acknowledgment to him before he had had the opportunity to publish the detailed studies. Mention is made of this because such magnanimity is not always appreciated, or is forgotten. Those of us who were recipients have not forgotten, but if we had forgotten, his other contributions would have won for him the same honor the Society is bestowing on him today.

Leon Herbert Schmidt was born in Huron, Ohio, the 28th of June, 1909. He received his baccalaureate degree from DePauw University with a major in chemistry in 1929. In the same year he entered the Graduate School of the University of Cincinnati as a Christ Hospital Fellow in Biochemistry. He was awarded the Doctor of Philosophy Degree in 1932. Following his graduation, he was appointed Research Associate, and became the first worker in the newly constructed Christ Hospital Institute for Medical Research, where he has remained until the present time. He has been Director of that Institute since 1936.

During his graduate study days Schmidt had worked on the fate of the bile acids in the animal body and he continued this work with diligence until 1936. His major achievement during this period was the demonstration that intestinal bacteria, particularly species of the genus *Alcaligenes*, were able to oxidize the OH groups of the bile acids in a stepwise manner to ketonic groups. This work on the bile acids led him into bacterial chemotherapy and indirectly pointed the way for his subsequent work in general chemotherapy. In 1936, at the beginning of the sulfonamide therapy cycle, he discovered that certain pneumococcal infections in mice were controlled by Prontosil S and Prontosil R. This finding, although contrary to the general belief at the time, nevertheless proved to be true and launched Schmidt and his coworkers on a program which for five years kept them in the forefront in investigations on sulfonamide drugs. During this period, one of his notable contributions concerned the renal toxicity of sulfa-

[1] Delivered at the annual meeting of the American Society of Tropical Medicine and Hygiene, New Orleans, Louisiana, October 31, 1956.

[2] Laboratory of Tropical Diseases.

merazine at the time when this drug was being considered a possible quinine substitute. This was his initial contribution to the malaria program of World War II.

The coordinated war-time search for more effective antimalarial drugs was uncovering compounds of sufficient interest to warrant trials in human subjects, but pharmacological studies which would provide data for effective use of the compounds were lacking; Schmidt stepped into this breach and became totally involved in the effort. His work centered around the 9-aminoacridines, the 4-quinolinmethanols, and the 4-aminoquinolines, studies which contributed considerably to the development of chloroquine.

While in mid-stream with this work, attention in the over-all program shifted to the 8-aminoquinolines because they seemed to offer possibilities for radical cure of vivax malaria. These compounds possessed the ability to bring about severe injury to the hemopoietic and central nervous system, a condition which could be detected with reliability only in man and a few subhuman primates. Because of his wide experience in handling the latter animals, he was asked to accept responsibility for evaluating these agents prior to human trial. The result was that by the end of World War II human trials with these compounds were largely dictated by the results of his work.

With the end of World War II many investigators in the over-all program returned to their peacetime interests, but for Leon Schmidt there was unfinished business in the field of malaria, and he began to employ *Plasmodium cynomolgi* in the monkey as a tool for studies on the biology and chemotherapy of this malaria. His basic work in this area forced attention on primaquine as the best of the potentially curative drugs, and set forth the therapeutic abilities and limitations of pyrimethamine. In addition, he carried on investigations which added much to our knowledge of the histopathology and the exoerythrocytic cycle of *P. cynomolgi*.

By late 1951 Dr. Schmidt decided to curtail his work on the chemotherapy of malaria and the following year launched a study to determine if it would be possible through the regular use of isoniazid to control established tuberculosis in a monkey colony and prevent its dissemination from infected to clean monkeys. The study was eminently successful in reducing or eliminating losses of experimental animals on his malaria chemotherapy project. But, as so often happens, the unexpected findings rather than the expected pointed the way for subsequent work. The above studies had shown that untreated simian tuberculosis is a striking counterpart to the pulmonary tuberculosis of young colored persons in the United States and of both young and old orientals. If this disease in the monkey were systematically reproducible, it would provide a valuable tool for studies on the biology and chemotherapy of tuberculosis. Consequently, since 1953 Dr. Schmidt has concentrated his efforts on the study of this disease with particular attention to the standardization of its response to known antituberculosis drugs and to problems on the pharmacology of isoniazid and cycloserine. The result is that human trials with these and other agents, combined on the basis of information accumulated in his pharmacological studies, are now under way in several different facilities.

In addition to his research and his duties as Director of the Christ Hospital Institute for Medical Research, Dr. Schmidt has found time for activities in many other directions. From his initial appointment as instructor in the Department of Biochemistry in the College of Medicine at the University of Cincinnati in 1932, he has moved through the ranks to the position of Research Professor, which he has held since 1950. He is Chairman of the Section on Pharmacology of the Committee on Growth of the National Research Council, and is a member of five other NRC committees. He is Consultant to the Surgeon General of the Public Health Service and Chairman of the Cancer Chemotherapy Study Section. He is a member of the Executive Committee of the Veterans Administration-Armed Forces Committee on Chemotherapy of Tuberculosis. He is a member of numerous scientific societies, the author of some 65 papers, and the recent recipient of the Eminent Chemist Award of the Cincinnati section of the American Chemical Society. His pre-eminence in his chosen field adds weight to his address this afternoon. I present the 21st Charles Franklin Craig lecturer, Dr. Leon Herbert Schmidt.

THE ROLE OF PHARMACOLOGY IN DETERMINING THE RATIONAL USE OF THERAPEUTIC AGENTS[1]

L. H. SCHMIDT

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Chemotherapy has often been described in terms of a triangle of two-way reactions involving host, parasite, and drug. Workers in tropical medicine have given considerable attention to the interactions of parasite and host and to the interplays between therapeutic agent and parasite. Mindful of safety, they have also given due consideration to the impact of the drug on the host. The reverse phase of this reaction, the effect of the host on the drug, has received far less attention despite the fact that it, as much as any and more than most reactions, determines therapeutic success or failure. It is this aspect of pharmacology, relating to the physiological disposition and metabolic fate of chemotherapeutic agents, which I would like to discuss in this the Twenty-first Lecture in honor of Charles Franklin Craig.

In developing this subject, I would like to give brief attention to those compounds which are used in the treatment and suppression of malaria, examining the extent to which present day usage is dictated by what the host does to a drug with respect to absorption, plasma binding, tissue localization, excretion, and metabolism. I would then like to turn in some detail to a subject less familiar to most of you, the metabolism of the antituberculosis drug, isoniazid, and show how knowledge acquired in this area is responsible for much of what is known today about the uses and limitations of the parent compound and its many derivatives.

At the outset of the discussion on the impact of host influences on current usage of antimalarial drugs, it is well to stress what is probably a basic requirement for the most successful treatment and suppression of human malaria. This is the continued maintenance of contact between parasite and effective concentrations of the therapeutic agent in plasma. Some may question the emphasis on this point in view of widespread acceptance of such a relationship as a general phenomenon. It is well to keep in mind, however, that associations between sustained plasma levels and therapeutic benefits were established for sulfonamides and penicillin in infections with certain Gram positive cocci but do not necessarily apply universally (Marshall, 1952). There are no definitive studies in human malarias which deal with this problem; however, all indirect evidence indicates that a sustained concentration of the antimalarial in plasma is required for maximum therapeutic benefit.

With this concept in the general background, let us now turn to consideration of the various drugs used in malaria therapy and suppression. Quinine, the oldest

[1] The Twenty-first Annual Charles Franklin Craig Lecture delivered at the Annual Meeting of the American Society of Tropical Medicine and Hygiene held in New Orleans, Louisiana on October 31, 1956.

specific chemotherapeutic agent, had an established position in malaria therapy long before the advent of modern pharmacology. Present day usage, however, is wholly consistent with what is known about absorption, excretion, tissue localization, and metabolism of the compound, a testimonial to the efficiency of long-term trial and error. Thus, quinine is rapidly absorbed, localized in tissue and bound to plasma to only slight degrees, metabolized extensively to inactive products, and excreted rapidly. One would predict that such qualities would make it necessary to administer any drug at frequent intervals if maximum activity is to be achieved. This is precisely the way quinine is administered in the treatment of human malaria when the severity of the disease requires achievement of maximum benefits from this drug. It is usually administered at 8-hour intervals even in mild infections and routine suppression, although in the latter situation, it is not uncommon to give it once a day.

The early therapeutic history of quinacrine provides an informative lesson on the importance of understanding the physiological disposition of a drug if its full potentialities are to be realized. For eight years subsequent to its discovery, quinacrine was administered in small divided daily doses as if it were the counterpart of quinine. This practice led to the impression that quinacrine was slow in action and probably inferior to quinine in the general therapy of malaria. The classical studies of Shannon and co-workers in 1942–43 (Shannon *et al.*, 1944) effected a radical change in this impression, and provided a rational basis for the effective use of this acridine in both treatment and suppression of malaria. These investigators found that quinacrine was rapidly absorbed, extensively localized in tissues and bound to plasma protein, and slowly excreted. When a dose of quinacrine was administered, it was deposited rapidly in certain tissues, then more or less slowly returned to plasma as the level in that fluid diminished due to metabolism and renal excretion. Shannon and co-workers promptly recognized the significance of these events and proceeded to fashion therapeutic and suppressive regimens. In the therapeutic regimen, use was made of short-term treatment with loading doses of quinacrine to saturate tissue reservoirs. The suppressive regimen involved use of weekly or bi-weekly doses. With these patterns of administration, the superiority of quinacrine over quinine became readily apparent.

The more recent additions to our antimalarial armamentarium, chloroquine, amodiaquine, chlorguanide, and pyrimethamine, resemble quinacrine fairly closely in so far as absorption, tissue localization, and excretion are concerned. This is now fairly well recognized in the case of chloroquine, amodiaquine, and chlorguanide, and will, I hope, be fully appreciated in so far as pyrimethamine is concerned after Dr. Smith has presented his findings to the Society (Smith and Ihrig, 1957).

The situation with respect to chlorguanide deserves special comment. Shortly after discovery of this drug, observations were made by Hawking (1947) and Hawking and Perry (1948) which suggested that chlorguanide owed its activity to conversion to an active metabolite. Similar observations were reported somewhat later by Geiman (1951). Not long thereafter, Carrington and co-workers

(1951, 1954) of Imperial Chemical Industries, and Crounse (1951) and Loo (1954) of our own Institute succeeded in isolating and characterizing this metabolite as a dihydrotriazine derivative. Biological tests with this derivative promoted immediate confusion. Against infections with *P. gallinaceum* in the chick, the metabolite was 10 to 50 times as active as the parent drug (Carrington *et al.*, 1951; Coatney, G. R., personal communication). Against infections with *P. cynomolgi* in the rhesus monkey, the parent drug was two to four times as active as the metabolite (Schmidt *et al.*, 1952). Studies in human volunteers also showed that the metabolite was less active than the parent compound (Davey, D. G., personal communication).

These findings posed two obvious questions. First, why is there a difference in the activity of the triazine metabolite in avian and primate malarias, and, second, why is the active triazine inferior to the parent inactive compound in human and simian malarias?

A likely answer to the first of these questions was soon to come from metabolic studies of Hughes (unpublished observations). These showed that when chlorguanide is administered to the chicken, less than 5 per cent is excreted as the active triazine. In contrast, man and monkey excrete more than 60 per cent of a dose of chlorguanide as the active metabolite. Thus in these latter subjects, direct administration of the triazine might contribute little since the compound is ordinarily formed when chlorguanide is ingested. On the other hand, the chicken would certainly benefit from administration of the triazine if as is indicated very little of the active metabolite is formed when the parent biguanide is administered.

The answer to the question of why the metabolite is inferior to the parent drug in primate malarias came from an unexpected source. While working on a problem related to the metabolism of a group of folic acid antagonists, two of my colleagues, Dr. Smith and Miss Fradkin (unpublished observations), had occasion to study the triazine metabolite of chlorguanide. They found that the urinary excretion of this compound was extremely rapid. Even a relatively massive dose of 40 mg. per kg. was cleared from the circulating blood completely in less than twenty-four hours. This finding, together with information on the tissue localization of chlorguanide, provides an explanation for the relative inefficiency of the active triazine and keynotes the contribution of the parent drug. This rapid renal elimination of the triazine metabolite by man and monkey makes it impossible to maintain the protracted contacts between drug and parasite which are essential to greatest therapeutic benefit when the drug is administered in a single large dose, or daily small doses. This contact is achieved when chlorguanide is administered in this manner because tissue localization of this compound insures a steady supply of precursor for conversion to the active metabolite.

With the background of this brief discussion on the role of the host in determining the use and efficacy of currently available antimalarial drugs, I would like to turn now to consideration of the metabolism of the antituberculosis drug, isoniazid, indicating how information in this area has been acquired and how such knowledge has influenced current use of this compound and its derivatives.

In developing this discussion, it will be necessary to draw freely on work which has been done at The Christ Hospital Institute of Medical Research, much of which is as yet unpublished. Most of this work has been carried out by Drs. Hughes, Biehl, the speaker, and their associates.

Shortly after introduction of isoniazid into therapy of human tuberculosis, our laboratory initiated a study designed to determine whether the hazards of pulmonary tuberculosis in a colony of rhesus monkeys could be eliminated by the prophylactic and therapeutic use of this drug. Needless to say, our interests at that time were not in tuberculosis per se but in maintaining a healthy monkey colony.

Definition of the pharmacological properties of isoniazid, including a study of metabolic fate, was an important feature of this investigation since it was anticipated that effective chemoprophylaxis would require long-term drug administration. This investigation demonstrated that isoniazid was rapidly absorbed, localized in tissue to only a slight degree, and extensively metabolized to products which were considerably less toxic than the parent drug and essentially devoid of activity against the tubercle bacillus (Schmidt *et al.*, 1953; Hughes, 1953). These products were acetyl isoniazid and isonicotinic acid. Their structures as related to that of isoniazid are set forth in Figure 1. This basic observation on the metabolism of isoniazid led to the development of quantitative methods of analysis and to collateral studies which showed that the monkey excreted 5 to 15 per cent of the isoniazid unchanged, 60 to 80 per cent as acetyl isoniazid, and the balance as free isonicotinic acid. As indicated in the figure, either isoniazid or its acetyl derivative could serve as the precursor of isonicotinic acid.

Explorations of the fate of isoniazid in other animal species, including man, soon followed. Only the observations in dogs and man are pertinent to the present

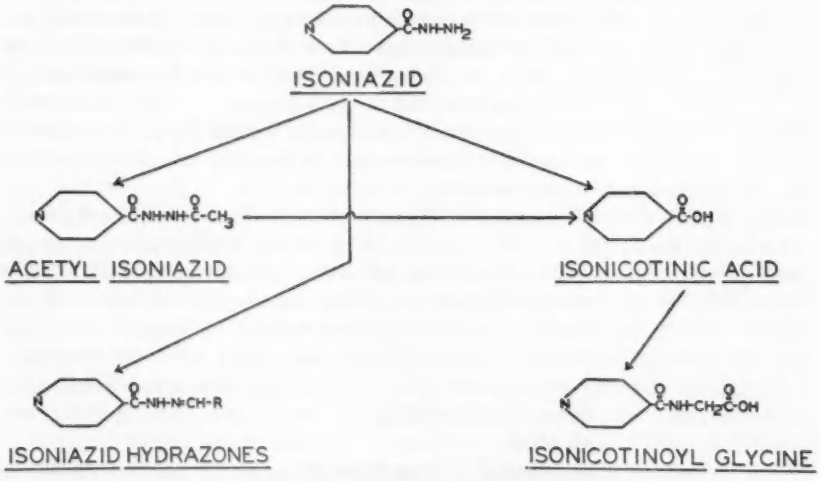

FIG. 1. Major metabolites of isoniazid

discussion. In man, the metabolism of isoniazid followed a pattern very similar to that noted in the monkey except that in addition to acetyl isoniazid and free isonicotinic acid, human beings excreted small amounts of conjugates of isonicotinic acid and variable amounts of a group of substances which yielded free isoniazid on mild acid hydrolysis (Hughes *et al.*, 1955). Because of their resemblance to certain known isoniazid derivatives, these latter materials have been called isoniazid hydrazones. Semi-quantitative analyses indicated that more than three-fourths of the isoniazid administered to human subjects was converted to these various metabolic products.

In the dog, the metabolism of isoniazid differed somewhat from that just described. As in the case of the sulfonamides (Marshall *et al.*, 1937a and b), this animal was unable to acetylate isoniazid. It eliminated 40 to 60 per cent of the parent drug unchanged, some 10 to 20 per cent as isoniazid hydrazones, and the balance as free isonicotinic acid.

These differences in the metabolism of isoniazid in man, dog, and monkey had several interesting implications. In the first place, they provided a rational explanation for the results of studies (Benson *et al.*, 1952; Rubin *et al.*, 1952; Sullivan *et al.*, 1954) which had indicated that isoniazid is considerably more toxic for the dog than for man or monkey. This difference could now be ascribed to the relatively poor metabolism of isoniazid in the dog and the fact referred to above that the metabolic products of this hydrazide have strikingly less toxicity than the parent drug. Secondly, the finding that the pattern of metabolism of isoniazid in man compared closely to that in the monkey raised a question as to the correctness of the currently recommended dosage regimen of isoniazid, which had been based largely on toxicity data acquired in the dog where the metabolic pattern was significantly different. This question became more pressing when it was recognized that maximum therapeutic benefits in simian pulmonary tuberculosis required administration of substantially larger doses of isoniazid than were then being employed in human tuberculosis (Schmidt, 1956) and that the response of the human disease to conventional doses of this drug was not uniformly satisfactory. This recognition led to an investigation of the therapeutic benefits of comparatively large doses of isoniazid in human pulmonary tuberculosis by Dr. Biehl of our Institute and a group of cooperating clinical investigators at a local tuberculosis sanatorium.

This study involved a group of 30 patients with far-advanced pulmonary tuberculosis which had resisted treatment with older antituberculous drugs and conventional doses of isoniazid. These subjects were given isoniazid in daily doses of 20 mg. per kg., approximately six times the dose which is usually employed. No untoward reactions to this high dose regimen were noted during the first four weeks of treatment. In the fifth week one patient developed symptoms of peripheral neuritis. Seven other patients developed similar reactions in the following eight weeks, after which the study was terminated (Biehl and Skavlem, 1953; Biehl and Nimitz, 1954).

This investigation contributed little to evaluating the therapeutic benefits of large doses of isoniazid. It did, however, serve as the forerunner of a study which

was to draw attention to the variations in metabolism of isoniazid in different human subjects and the relations between these variations and peripheral neuritis. It also led to an effective procedure for preventing the occurrence of this untoward reaction.

The study precipitated by the initial observations was stimulated by Dr. Vilter and associates of the University of Cincinnati, College of Medicine, who had just succeeded in inducing peripheral neuritis by administration of the pyridoxine antagonist, desoxypyridoxine (1953). Vilter recognized certain similarities between this syndrome and that evoked by large doses of isoniazid, and suggested an experiment designed to determine whether the administration of such large doses affected the excretion of pyridoxine, pyridoxal, or pyridoxamine, or produced those changes in the metabolism of tryptophane usually associated with vitamin B_6 deficiency. Positive answers were obtained to both of these questions (Biehl and Vilter, 1954a and b). These led to the obvious next step of determining whether the appearance of peripheral neuritis could be prevented by the simultaneous administration of pyridoxine. This was accomplished successfully (Biehl and Vilter, 1954b).

As the above studies progressed, questions were raised as to why only a limited fraction of subjects developed peripheral neuritis while receiving large doses of isoniazid. It occurred to some of us that this phenomenon might be related to the metabolism of isoniazid. The availability of quantitative daily urine collections on the group of subjects used in the B_6 metabolism study, some of whom had developed peripheral neuritis and some not, provided material for testing this suggestion. Systematic analyses on the urine of these subjects revealed wide quantitative differences in the elimination of isoniazid and its metabolites, and more important, a direct relationship between the occurrence of peripheral neuritis and the excretion of the parent drug (Hughes *et al.*, 1954).

These findings seemed to have important implications for the therapeutic activity of isoniazid as well as for its toxicity. Limited studies had indicated that the distribution of isoniazid and its metabolites in urine clearly reflected the distribution of these substances in blood and other body fluids. Assuming the correctness of this observation, one could inquire whether the subject who metabolized isoniazid extremely well and had little of the free, microbiologically active drug in body fluids could be expected to gain the same therapeutic benefit from a given dose as a patient who metabolized the compound poorly with resultant high levels of active drug.

Before approaching this question, an attempt was made to determine whether the wide variations in the metabolism of isoniazid in different individuals were peculiar to the use of high doses of this drug or whether they were also common to conventional doses. An answer to this question was sought in a group of 61 patients with pulmonary tuberculosis who were receiving isoniazid in daily doses of 3 to 5 mg. per kg. along with 1 gram of streptomycin twice weekly. Systematic studies in these subjects revealed the same wide variations in total metabolism of isoniazid as were noted in patients receiving doses of 20 mg. per kg. The extent of these variations as reflected in the excretion of free isoniazid has been sum-

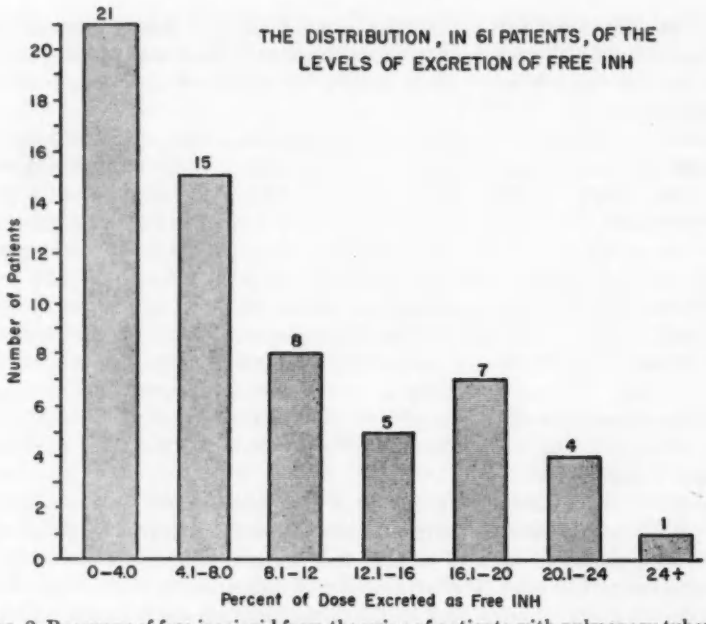

FIG. 2. Recovery of free isoniazid from the urine of patients with pulmonary tuberculosis (Daily dose—3 to 5 mg. per kg. body weight).

marized in Figure 2. The data therein show that approximately one-third of the patients who received 3 to 5 mg. per kg. doses excreted less than 4 per cent of the dose of isoniazid unchanged; approximately another third eliminated from 4 to 12 per cent, while the final third excreted 12 to 24 per cent.

Metabolic studies on normal human volunteers gave somewhat similar findings. The results of a typical experiment are summarized in Table 1, the second column of which shows that the output of unchanged isoniazid in these normal individuals varied from 1 to 17 per cent.

Additional investigations on both normal and tuberculous subjects have demonstrated that the pattern of isoniazid metabolism for any given individual is remarkably constant over a period of at least a year. It is worth noting that the metabolic pattern appears to be independent of the state of debility or intoxication.

With this background it seemed reasonable to approach the question of the relation between the metabolism of isoniazid and the therapeutic effectiveness of this drug in human pulmonary tuberculosis. A study of this question was started more than a year ago and will continue for some time. Only one aspect of the investigation will be reported here, that which deals with the relation between the excretion of free isoniazid in the urine and the appearance in the sputum of tubercle bacilli resistant to at least 1γ of isoniazid per ml. of medium, an event which is regarded by some as evidence of treatment failure. Figure 3 summarizes

TABLE 1

The metabolism of isoniazid in normal human subjects
(Single dose—5 mg. per kg.)

Subject and sex	Per cent of dose recovered in urine					
	Free isoniazid	"Isonicotinoyl hydrazones"	Acetyl isoniazid	Isonicotinic acid	"Isonicotinic acid conjugates"	Total
D—F	1.0	8.3	60.1	34.3	0	103.7
AG—M	3.0	9.2	59.1	35.6	0	105.9
LS—M	4.1	3.0	56.4	35.0	0	98.1
CS—M	4.8	7.4	55.2	35.0	0	102.3
AA—M	5.1	3.0	61.7	23.1	5.3	98.2
RF—F	8.1	13.4	40.3	27.0	15.2	104.0
HH—F	10.3	8.0	38.9	27.6	15.6	100.4
RH—M	10.8	11.3	40.1	23.3	7.5	93.0
RA—M	15.0	12.4	30.4	24.6	23.6	106.0
HE—F	16.2	11.8	39.8	22.4	16.2	106.4
GS—F	16.8	11.2	36.7	22.0	13.6	101.3

findings obtained on a group of patients who have completed 6 months treatment with daily doses of 5 mg. of isoniazid per kg. body weight and 1 gram of streptomycin twice weekly. It will be noted that isoniazid-resistant tubercle bacilli were found in the sputa of 8 of the 17 patients who eliminated less than 4 per cent of the drug unchanged. Such bacilli were found in only 3 of the 27 patients who eliminated larger fractions of the microbiologically active drug. Whereas these data do not prove that the pattern of isoniazid metabolism is the only factor involved in the emergence of isoniazid-resistant tubercle bacilli, they suggest that it is an important influence.

The observations presented to this point have dealt largely with the manner in which knowledge of the metabolic fate of isoniazid has influenced use of the parent compound. Before concluding this discussion, I would like to give you some idea of the manner in which knowledge of isoniazid metabolism can guide the use of isoniazid derivatives. Such a guide is almost a priceless tool in the chemotherapy of tuberculosis where the wide variations in the natural course of the disease and the long periods of observation required for definitive studies make critical evaluations of new therapeutic agents especially difficult.

Invariably when a new derivative of isoniazid is introduced, the first question raised is whether this compound owes its activity to the liberation of isoniazid or to the intact molecule. In the latter event it may have unique benefits; in the former it may be no more than an expensive and inefficient way of administering isoniazid. Fortunately the consistency of the metabolism of isoniazid in a given individual makes it possible to examine the above question with comparative ease.

I would like to illustrate this point by reference to a recent study carried out in our laboratory on a compound called Hinconstarch, which is a condensation product of isoniazid, p-aminobenzaldehydethiosemicarbazone, and oxidized

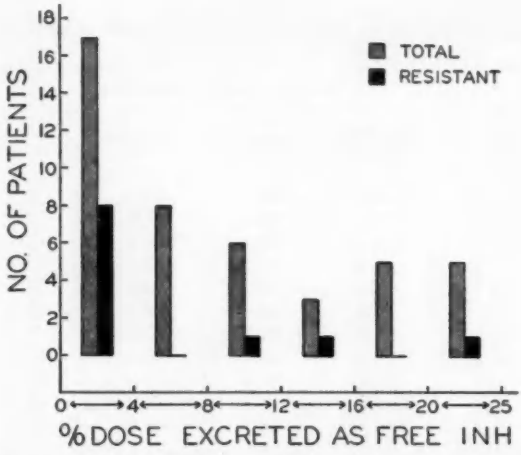

FIG. 3. The emergence of tubercle bacilli resistant to isoniazid as related to the metabolic fate of this drug (Patients receiving 3 to 5 mg. isoniazid per kg. body weight).

TABLE 2

Excretion of isoniazid and its derivatives following ingestion of isoniazid or Hinconstarch
Dose—2.5 mg. of isoniazid per kg. body weight or Hinconstarch equivalent

Subject	Compound ingested	Per cent of dose excreted	Per cent of recovered drug as			
			Isoniazid	Isoniazid hydrazones	Acetyl isoniazid	Free isonicotinic acid
HH	INH	95.4	21.3	15.5	35.7	27.5
	HCS	19.9	25.1	20.1	28.2	26.6
LS	INH	105.0	5.5	1.5	56.1	36.9
	HCS	22.0	6.9	1.0	55.4	36.7

starch. This compound and an equivalent amount of isoniazid were given on alternate days to two human volunteers who collected their urine at various intervals over each twenty-four hour period. These urine specimens were analyzed for content of unchanged Hinconstarch, isoniazid, and its derivatives. The results of this study, condensed in Table 2, lead to two conclusions: first, that as compared with isoniazid, Hinconstarch is poorly absorbed; and, second, that the fraction which is absorbed is metabolized precisely as is isoniazid. Such an observation, which probably justifies the conclusion that Hinconstarch is unlikely to have any therapeutic advantage over isoniazid, can be made in a matter of a week and saves months or even years of time spent in difficult-to-control clinical trials.

With these observations I would like to bring to a close this discussion of the role of the host in determining the activity of therapeutic agents. The influence

of such factors as absorption, metabolism, excretion, and tissue distribution on the use of currently available antimalarial drugs has been summarized. The manner in which knowledge of the metabolism of the antituberculosis drug, isoniazid, has been acquired and how this knowledge has influenced and is influencing the use of this agent has been dealt with at some length. The fact that in some instances usage of a drug was not arrived at through conscious recognition of such host influences as the above should not detract from the importance of defining such influences. The least that such studies can contribute is a shortening of the time table required to effect most efficient use of a therapeutic agent. The most they can do is to bring to light pertinent facts which if not available and exploited could lose to chemotherapy the services of a valuable agent.

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THE RESPONSE OF *PLASMODIUM MALARIAE* INFECTIONS TO PYRIMETHAMINE (DARAPRIM)

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The comparative effectiveness of various antimalarial agents against induced infections of *Plasmodium malariae* (USPHS strain) has been reported previously (Young and Eyles, 1948; McLendon and Young, 1951). The present report gives the response of this malaria to pyrimethamine (Daraprim), one of the newer drugs.

METHODS

The patients were receiving malaria for the treatment of neurosyphilis. One infection was induced by the bites of infected mosquitoes, the others by the intravenous inoculation of infected blood. Blood smears were examined daily and parasite densities determined by counting the parasites in at least 0.1 cmm. of blood.

The drug was usually given late in the primary attack, after the patient had experienced many fevers, and several weeks after the maximum parasitemias. It was administered as a single daily dose for either one or two days.

RESULTS

Fifteen patients received a total of 17 treatments with pyrimethamine. The results are summarized in Table 1. Fourteen patients were symptomatic when treatment was begun; eight of these experienced one or more fevers after drugging. Three of the patients were not cleared of erythrocytic parasites; in the

TABLE 1

Response of Plasmodium malariae *infections to pyrimethamine*

Drug				Parasites										Relapses	
Daily mgm.	No. days	No. Patients	Av. No. Fevers	Av. no. per cmm.	Days after beginning of treatment							Days to clearance		No./ followed	Range days
					1	2	3	4	5	6	7	Range	Median		
					Cumulative percentage of parasites removed										
25	1	9	17	5545	17	42	73	81	96	97	98	5–15	13	5/6	28–54
50	1	2r	17	1440	inc.	inc.	23	22	39	4	47	c.p.	c.p.	—	—
50	1	2	24	1444	inc.	25	59	95	97	97	98	12–14	13	0/2	—
50	2	4	18	2821	28	81	82	93	94	98	98	9–12*	11	2/3	17–36

r—These patients were relapsing patients from the above group.
inc.—Parasites increased.
c.p.—Continuous parasitemia until retreated.
* One not cleared.

remaining 14, the range of clearance time was five to 15 days with a median of 12 days. The patient with the quickest clearance of five days had only 160 parasites per cmm. and was asymptomatic at the time of treatment.

Between the three regimens of 25, 50, or 100 mgm., there was very little difference in the rate of clearance of erythrocytic parasites in the groups exposed to the drug for the first time. It is interesting to note that the poorest response to the drug was in two patients (Table 1, second line) who had taken the drug previously. Although the second dose was twice that of the first dose, the reduction in parasite density was slower than after the first exposure and the erythrocytic parasites were not cleared from the blood stream.

The relapse rate was similar for the 25 and 100 mgm. regimens. The total relapse rate was high; seven of 11 patients showed parasite relapses with a median of 36 days.

It is apparent from the data that pyrimethamine is a relatively slow acting schizonticide against *P. malariae* infections. It is less effective than chloroquine given by mouth or intramuscularly and appears to act about as fast as quinine against this strain of malaria (Young and Eyles, 1948; McLendon and Young, 1951).

SUMMARY

Pyrimethamine was tried 17 times against *Plasmodium malariae* (USPHS strain) infections in 15 neurosyphilitic patients. The drug was given on one or two days in amounts totaling 25, 50, or 100 mgm. The response of the erythrocytic parasites was comparatively slow with a median of 12 days to clearance in 14 patients. Of 11 patients with an adequate follow-up, seven relapsed. Two patients who had relapsed after 25 mgm. were treated with 50 mgm. The latter dose failed to eliminate the erythrocytic parasites.

ACKNOWLEDGEMENTS

Appreciation is expressed to Mr. Jimmie C. Skinner for technical aid, to the Staff of the South Carolina State Hospital for cooperation, and to Burroughs Wellcome Company, Tuckahoe, New York, for furnishing the pyrimethamine (Daraprim®).

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NOTE ON THE TRANSFER OF STRAINS OF *ENTAMOEBA HISTOLYTICA* FROM ONE BACTERIAL FLORA TO ANOTHER[1]

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Certain experiments have recently been designed in which it was desirable to transfer specific strains of *Entamoeba histolytica* from the Shaffer-Frye (S-F) medium to a diphasic medium with the mixed bacterial flora of another strain of *E. histolytica*. In order to accomplish this it was necessary to separate the particular flora to be used from its accompanying strain of amebas. It was routine practice to take a loopful (4 mm.) of inoculum from the top of the fluid column of the culture of *E. histolytica* in diphasic medium and transfer it to a similar medium. Since the multiplication of the amebas in such a culture occurs in the bottom of the fluid phase, it seemed unlikely that any amebas would be carried over with a loop transfer taken from the top. It was therefore surprising to find that in a high per cent of such transfers *E. histolytica* was present when the cultures were examined after 24 to 48 hours incubation at 37° C.

TABLE 1

Results of transfer of loopful of fluid from the top of the fluid phase of E. histolytica *cultures in mixed bacterial flora*

Strain of *E. histolytica*	Transfer*			
	Positive	Negative	Total	Per cent positive
Huff	50	10	60	83
Shelton	50	10	60	83
NRS	33	27	60	55
103	25	34	59	42
Parker	23	36	59	39
Conrad†	0	60	60	0

* Each transfer was made from a separate culture and the whole series covers a period of three months.

† Small race.

Following this observation, experiments were set up in which loop transfers were made repeatedly from 24, 48 or 72-hour cultures of 6 different laboratory strains of *E. histolytica* and the per cent of positive *E. histolytica* cultures from each strain calculated. The cultures for transfer were incubated in a vertical position and left completely undisturbed until the transfer was to be made. They were carefully removed from the incubator and the loopful taken with a

[1] This investigation was supported by a grant from the Microbiological Institute (E-499C_3) of the National Institutes of Health, Public Health Service.

minimum of handling in order to avoid any possibility of unduly mixing the organisms. The results of these experiments with the 6 strains of *E. histolytica* are summarized in Table 1.

The results shown in Table 1 reveal that there was considerable variation in the per cent of positive loop transfers with the various strains. No positive transfers were obtained with the Conrad strain, but the others yielded from 39 to 83 per cent positives. This is of some interest since the Conrad strain was the only small-race strain used in the series. This strain was much less motile than the large-race strains. Other experiments have shown that it takes large numbers of Conrad amebas to initiate a culture. Either or both of these characteristics may explain the differences in findings.

During the course of the above experiments, loopfuls of culture, taken as described for transfer, were placed on slides and examined microscopically. Actively motile trophozoites were very frequently seen in such preparations, especially with the Huff and Shelton strains, but not with the Conrad strain.

In another experiment, an attempt was made to see how long the Shelton and Huff strains could be maintained by loop transfer. The Shelton strain was lost after the 10th subculture, but the Huff strain was maintained through 25 transfers at which time it was discontinued.

COMMENT

A ready explanation can not be given for the occurrence of *E. histolytica* trophozoites at the top of the fluid phase in these cultures. It is possible that they migrate up the slant and float off into the medium, but a better explanation may be that they are carried up from the bottom by convection currents or by gas bubbles resulting from the metabolic activity of the accompanying bacteria. It would seem unlikely that any multiplication of the amebas would occur so near the top of the culture.

These results emphasize the importance of extreme care in all experiments involving the shift of *E. histolytica* from one medium containing associated organisms or flora to another. One must be absolutely sure that the flora into which the inoculation is being made is completely free from the *E. histolytica* with which it was originally associated.

VISCERAL LEISHMANIASIS IN A DOG IMPORTED INTO THE UNITED STATES

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Recently, Thorson *et al.* (1955) reported a case of visceral leishmaniasis in a dog that had been imported into the United States from Greece. This, these authors claimed, was the first such case in the United States. The purpose of this report is to record another instance of visceral leishmaniasis in a dog which had also been imported into the United States from Greece.

HISTORY

Two littermate boxer pups, born in Germany, were shipped to Greece at the age of six weeks. They remained in a suburb of Athens for approximately three years. They were then shipped back to Germany by air and were boarded in a kennel in Frankfurt. Approximately one month after they arrived in Germany, both dogs became ill and were brought to a veterinarian. A diagnosis of distemper was made and the dogs were hospitalized. Both dogs were febrile; one exhibited "chewing fits" on the second day of hospitalization. The diagnosis was then changed to "brain distemper" and a guarded prognosis was given. Both dogs showed central nervous system involvement manifested by incoordination and paresis. They were treated with antibiotics and supportive therapy. One dog became progressively worse, developing blindness and paralysis of the hind quarters, until it died exactly one month from the onset of the illness. The other dog developed paralysis of one hind leg from which it apparently recovered but whether recovery was complete is not clear. The surviving dog was shipped by air to the United States, arriving in New York approximately six weeks after the illness was first detected in Frankfurt. A "loss of balance" was noted immediately after arrival—a condition from which there was again apparent recovery. The dog, however, continued to be in poor condition and was intermittently hospitalized in Washington, D. C., until euthanasia was performed three months after it arrived in the United States.

The subject was first seen by one of the authors (I. C.) two-and-one-half months after its arrival in the United States. At this time it was febrile (102.5°F.), thin but not cachectic, exhibited a yellowish nasal discharge and marked hyperkeratosis of the nostrils. Several bursae were found to be enlarged and a serous discharge exuded from some of them. The right inguinal, mandibular, pharyngeal, and left and right popliteal lymph nodes were enlarged. Considerable edema was located in the lower tibial region of the right leg, and the bursitis mentioned earlier was noticeably more severe on the right side in the fore and hind legs. Homologous anti-distemper-hepatitis serum and chlortetracycline were administered. Two days later 50 mg. of cortisone and 10 mg. of desoxycorticosterone

were administered. The general condition of the dog deteriorated, the edema became more pronounced, the breathing became more difficult, and physical weakness became evident. Euthanasia was performed at the request of the owner. A few days prior to this, however, a blood specimen, and a bone-marrow biopsy were obtained for culture. The right popliteal lymph node was also extirpated. At this time pronounced delay in blood-clotting time was observed.

CULTURAL STUDIES

Specimens of blood, bone marrow, and lymph node were cultured immediately upon receipt in the laboratory on slants and in the water of condensation of NNN (Novy, MacNeal and Nicolle) medium. Citrated blood was also cultured in Fletcher's semi-solid medium. All cultures were incubated at room temperature, 22–24°C. Cultures were examined under light and darkfield microscopy and smears were also examined after being fixed and stained with Giemsa's stain. Nine days after inoculation the bone marrow cultures contained the leptomonad forms of *Leishmania sp.* These forms were observed in the Giemsa stained preparation and as motile organisms under the microscope. Subcultures into NNN medium proved to be positive for the leptomonad form in three days, (Fig. 1). Twenty days after being inoculated all cultures of the blood and lymph nodes were negative.

When the cultures were originally prepared, 25–30 gm. hamsters were also inoculated intraperitoneally with a suspension of macerated bone marrow. No isolations were made from these hamsters, but the leptomonads recovered from the NNN medium were successfully passed through hamsters four times by intraperitoneal injection of ground hamster liver material. Parallel passages were made of the cultured organism on the chorioallantoic membranes of embryonated chicken eggs.

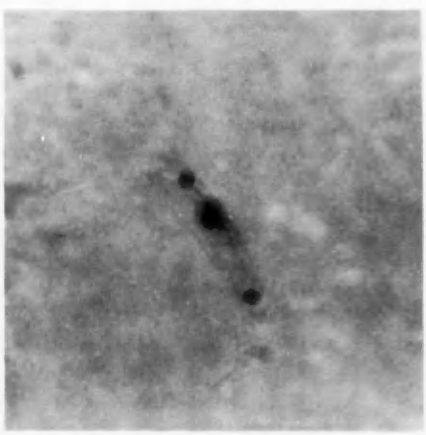

FIG. 1. Leptomonad form of *Leishmania* in bone marrow subculture, NNN medium, illustrating the centrally located nucleus and the parabasal bodies. (×2240) (AFIP Neg 56-9029).

CLINICAL PATHOLOGIC STUDIES

Serum from noncitrated blood was tested for euglobulin using Napier's aldehyde test. The serum became opaque, and coagulated within a few minutes—a positive presumptive test for kala-azar. The plasma protein content was found to be 13 per cent, considerably higher than normal for dogs (4–6%). The results of an electrophoretic analysis of the plasma are summarized in Table 1 and Figure 2.

When the sample of serum was cooled to 4°C., a copious white precipitate formed and the sample congealed into a gel. When the temperature was raised to 37°C. the sample re-liquified and the precipitate dissolved (the presence of "cold-susceptible" proteins in the sera of dogs with leishmaniasis has previously been noted (Stein and Wertheimer, 1942).

The electrophoretic analysis of the serum sample was performed in a Perkin-Elmer Model 38 electrophoresis device using a diethylbarbiturate (veronal) buffer at pH 8.6, ionic strength 0.10. The striking abnormalities noted were: (1) a pronounced hypoalbuminemia; and (2) extraordinarily high elevation of the gamma globulin fraction which appeared in the pattern as two separable peaks. Presumably these are the proteins which precipitate in the cold. Similar,

TABLE 1

Composition of the serum as determined by electrophoresis in veronal buffer at pH 8.6, ionic strength 0.10

Component	% of total protein*	g./100 ml. of serum
Albumin	5.5	0.7
α-globulins	6.0	0.8
β-globulins	5.2	0.7
γ-globulins	83.3	10.8
Total	100.0	13.0

* By measurement of the area of the ascending pattern.

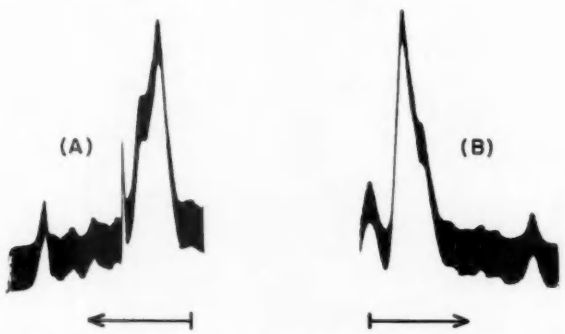

FIG. 2. Descending (A) and ascending (B) electrophoretic patterns of the serum after migration for 84 minutes in veronal buffer, pH 8.6, ionic strength 0.10, at 1° C. Serum dilution 1:4; current 0.015 ampere. Total protein concentration was 13 g. 100 ml. of serum.

although less extreme, variations in human kala-azar have been reported by Lloyd (1928), Cooper (1946), Chakravarti (1950), and Sen Gupta *et al.* (1953).

PATHOLOGIC STUDIES

Most unfortunately, a complete autopsy was not performed. The abdominal wall, however, was incised and a few sections of viscera were selected. The viscera appeared to be in normal relationship to each other, and the spleen and liver did not appear to be significantly enlarged. Microscopic examination of the sections stained with hematoxylin and eosin revealed the following abnormalities.

Kidney. Cortical scars extending down into the medulla were observed. They were infiltrated heavily with lymphocytes. Heavy perivascular lymphocytic infiltration was also observed.

Lung. The majority of the alveoli were filled with pink homogeneous amorphous material. No inflammatory processes were recognized.

Liver. There was a heavy infiltration of lymphocytes and plasma cells into the hepatic triads. Many of the Kupffer cells lining the sinusoids were filled with hemosiderin. There was no frank necrosis of the hepatic parenchyma; however, there appeared to be a loss of cytoplasm in many of the hepatic cells which varied in their staining characteristics. The hepatic cords also seemed to be separated and atrophied.

Lymph node. There was a marked hyperplasia of lymphocytic and reticuloendothelial cells. The normal histologic architectural pattern of the lymph node was obscured by the proliferating elements. Some sections contained considerable hemosiderin. Leishmania were easily found in the proliferating reticuloendothelial cells, which probably phagocytized the organisms.

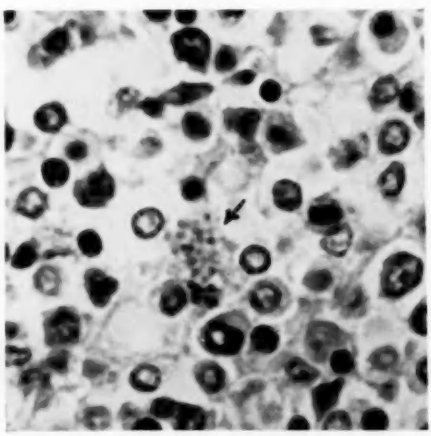

FIG. 3. Bone marrow (×1100) *Leishmania* in reticulo-endothelial cell. (AFIP Neg No 56-9026).

Bone marrow. Figure 3 shows a section of a highly cellular marrow in which many megakaryocytes and a proliferation of the reticulo-endothelial elements can be seen. Leishmania are easily found within these reticulo-endothelial cells. A Periodic Acid Schiff preparation with a light green counterstain differentiated the organisms from *Histoplasma capsulatum.*

SUMMARY

A second case of visceral leishmaniasis in a dog imported into the United States from Greece is presented. Diagnosis was substantiated by demonstrating the presence of the organism in cultures of tissue removed from this animal and by histologic examination. The leptomonad forms were cultured from sternal bone marrow on NNN medium. The introduction of active visceral leishmaniasis into the United States in an animal host poses a problem which should properly concern public health and animal disease control officials.

This case has been contributed to the Registry of Veterinary Pathology and has received AFIP Accession No. 750721.

ACKNOWLEDGMENTS

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ISOLATION OF YELLOW FEVER VIRUS FROM *HAEMAGOGUS MESODENTATUS*, *H. EQUINUS* AND *SABETHES CHLOROPTERUS* CAPTURED IN GUATEMALA IN 1956

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A wave of sylvan yellow fever has been progressing steadily northward in Central America in recent years. The first cases since 1905 were identified in Panama in the late fall of 1948 (Herrera *et al.*, 1949). Since that time, outbreaks have appeared successively in Costa Rica, Nicaragua and Honduras. Data concerning these outbreaks have been reviewed by Soper (1955). Near the beginning of the year 1956 the northward sweep reached Guatemala, and arrangements were made by the Pan American Sanitary Bureau with the Gorgas Memorial Laboratory to attempt virus isolations from mosquitoes captured in areas showing yellow fever activity. The staff of the Gorgas Memorial Laboratory has been interested in the problem of the Central American vectors of the disease for a number of years. Trapido and Galindo (1955) and Galindo and Trapido (1956) have shown that the principal South American vectors, *Haemagogus spegazzinii* together with its subspecies *falco*, and *Aedes leucocelaenus*, are scarce or do not occur at all in certain areas of Central America where sylvan yellow fever outbreaks have appeared. In 1949, an attempt was made by one of the authors (Rodaniche, 1951) to recover yellow fever virus from mosquitoes captured in the Pacora-Buena Vista sector of Panama with negative results. A similar attempt was made again in 1954 in connection with an outbreak of sylvan yellow fever in Honduras. *Haemagogus* captures were small, however, and findings again were negative for yellow fever virus (Rodaniche, 1956). During the recent Guatemalan outbreak, Drs. J. Boshell Manrique and George Bevier, who were engaged in field epidemiological studies there for the P.A.S.B., arranged to ship mosquitoes to the Gorgas Memorial Laboratory for possible virus isolations. Collecting stations were established by the P.A.S.B. in the Motagua valley of Guatemala in two principal zones; Rio Blanco, where simian deaths were first noted in February 1956, and El Tipon, where epizootic activity was first observed in May of the same year. Shipments from Rio Blanco were begun in March, and from Tipon in May, and have been continued up to the present time (October) from both locations. Details of the epidemiological studies conducted by the Pan American Sanitary Bureau are to be reported by Drs. Boshell and Bevier. In the present report, we shall deal only with the yellow fever virus isolations effected in the Gorgas Memorial Laboratory.

The overwhelming majority of diptera received were Culicidae. Exceptions are noted in Table 1. Shipments were made by plane, the mosquitoes being stored in screw-capped bottles lined with plaster of Paris and contained within plastic bags in ice-filled thermos jugs. The time elapsing between capture and receipt at the Laboratory never exceeded 4 days and the mosquitoes frequently

TABLE 1

Yellow fever virus isolations from diptera collected in the Motagua Valley, Guatemala, from March to October, 1956*

Species	Number of specimens received	Number of virus isolations
Haemagogus equinus	1,668	3
Haemagogus mesodentatus	4,021	14
Sabethes chloropterus	3,141	4
Sabethes cyaneus	163	0
Sabethes tarsopus	73	0
Sabethes spp.†	30	0
Aedes angustivitattus	18	0
Aedes scapularis	2	0
Aedes serratus	1	0
Aedes terrens	9	0
Aedes (Ochlerotatus)	2	0
Psorophora cingulata	2	0
Psorophora ferox	7	0
Psorophora lutzii	369	0
Trichoprosopon leucopus	1	0
Trichoprosopon magnum	1	0
Trichoprosopon spp.	24	0
Wyeomyia	289	0
Mansonia indubitans	2	0
Mansonia titillans	1	0
Anopheles eiseni	1	0
Anopheles neivai	1	0
Anopheles punctimacula	1	0
Chagasia spp.	21	0
Chagasia bathanus	15	0
Simulium sp.	10	0
Phlebotomus sp.	1	0
Culicoides sp.	1	0
Total	9,893	21

* Captures were made by Drs. J. Boshell-Manrique and G. Bevier of the P.A.S.B.
† Not including *Sabethes chloropterus*.

were still active on arrival. They were classified according to species and any obviously engorged specimens were discarded. However, a number of mosquitoes were found to contain small to moderate quantities of fresh blood on trituration. It was not considered practical under field conditions to maintain the mosquitoes for 12 or more hours at environmental temperatures in order that any recent blood meal might be digested before storing them in ice for shipping.

MATERIALS AND METHODS

In the virus section of the laboratory, the mosquitoes either were used at once for mouse inoculation or were stored in sealed tubes in dry ice until such time as inoculation into mice was feasible. Each mosquito pool was triturated in a sterile mortar and then emulsified in 10 per cent rhesus-serum saline con-

taining 1,000 units of potassium penicillin G and 1,000 micrograms of dihydrostreptomycin per cubic centimeter. In general, one cc. of diluent was used per 50 mosquitoes and roughly proportionate quantities for larger or smaller pools. However, 0.5 cc. was considered minimal.

The number of mosquitoes used as inoculum for each mouse group, varied from one for the rarer species, to 163 in the largest pool employed. Size of pools was governed by several factors; the number of specimens of each species or species group received, the place of capture and the time of capture. At the beginning of the study, the more abundant species were pooled in groups of approximately 50. Smaller pools were preferred as giving more epidemiological information. Later in the course of the study, larger pools were made wherever feasible to conserve time and materials.

After centrifugation at 1,500 r.p.m. for 10 minutes at a temperature of 4°C., the supernatant solution was injected intracerebrally into groups of 6 white Swiss mice, each mouse receiving 0.03 cc. Young adult mice 21 to 30 days of age were usually employed, as sufficient infant mice were not available. Infant mice were occasionally used for special procedures. Mice were kept under daily observation for a period of 30 days. Any showing suspicious symptoms were sacrificed and examined postmortem for macroscopic pathology. Their brains were removed aseptically and impression smears stained with Giemsa and cultures on blood agar plates and in thioglycollate broth were prepared. Then the brains were suspended in physiological saline solution for subinoculation in mice.

IDENTIFICATION OF YELLOW FEVER VIRUS

Identification of yellow fever virus was based on cross-immunity and mouse-protection tests. For cross-immunity tests, mice surviving original inoculation with mosquito suspensions or successive subinoculations, were challenged with 10,000 LD/50 of French neurotropic yellow fever virus. Results were considered positive if these mice remained healthy after all controls had died. In reverse cross-immunity tests, virus recovered in mice from infected mosquitoes was inoculated intracerebrally into groups of 6 mice previously immunized against the French neurotropic yellow fever virus and into 6 normal controls. Results were considered positive if all the yellow-fever-immune mice remained healthy after the normal mice had sickened or died. In addition, an intraperitoneal mouse protection test employing a known yellow-fever-immune and a known negative monkey serum, according to the technique developed by Sawyer and Lloyd (1931), was conducted for each virus strain isolated. Survival of at least 5 of the 6 mice receiving the immune-serum virus mixture and death of at least 5 of the 6 mice injected with the negative serum-virus mixture after an incubation period of at least 4 days was interpreted as a positive reaction.

For the first isolation of yellow fever virus from *Haemagogus mesodentatus*, a night monkey and a marmoset, previously tested and found free of antibodies to yellow fever, were inoculated subcutaneously with first mouse passage virus. Circulating virus was recovered from the blood of these monkeys on the fourth

day after injection. The animals died on the sixth and ninth days after inoculation respectively, and the diagnosis of yellow fever was confirmed by Dr. Carl M. Johnson by histopathological examination.

RESULTS

A total of 9,893 diptera were used for virus isolation attempts during the course of these studies. The great majority (8,848) belonged to the species *Haemagogus mesodentatus* with 4,021 specimens, *H. equinus* with 1,686, and *Sabethes chloropterus* with 3,141 (Table 1). Yellow fever virus was recovered 14 times from pools of *Haemagogus mesodentatus*, 3 times from *H. equinus* and 4 times from *Sabethes chloropterus*. No virus isolations were made from any of the other species.

Pools of *H. mesodentatus* from which yellow fever virus was recovered varied from 35 to 163 specimens with an average of 77. Eleven isolations were made from 2,412 specimens received from Rio Blanco from March to May and 3 isolations from 1,609 specimens received from El Tipon in June and July. Pools of *H. equinus* yielding virus contained 31, 51, and 136 specimens respectively. One isolation was made from a pool of this species captured at Rio Blanco in March and April and one from a pool captured in the same area in May. The third isolation was from specimens captured in El Tipon in June. A total of 328 *H. equinus* were obtained from Rio Blanco and 1,358 from El Tipon. All isolations from *Sabethes chloropterus* were made from specimens captured in May, three being from pools of 78, 122, and 122 mosquitoes respectively, from Rio Blanco where a total of 2,527 specimens was captured, and one from a pool of 47 specimens from El Tipon where 614 specimens were obtained.

The average incubation period in mice on primary isolation was 13 days, the shortest 7 days, and the longest 25 days. Virus isolates showed somewhat variable virulence for mice on primary isolation, some requiring several intracerebral passages before death of all animals in the inoculated groups ensued.

One group of mice inoculated with the pool of 78 *Sabethes chloropterus* yielded a mixture of two viruses. Yellow fever virus was recovered in pure state by inoculation of a night monkey and a marmoset both of which showed circulating virus on the fourth day. The other component of the mixture has not yet been identified. Two other neurotropic viruses, shown by immunological studies not to be yellow fever virus, also were recovered in the course of these studies.

DISCUSSION

Whitman (1951) has reviewed work incriminating *H. spegazzinii* and its subspecies *falco* as the principal vectors of sylvan yellow fever in Brazil and Colombia respectively. Yellow fever virus recently was isolated repeatedly in Trinidad from pools of *Haemagogus spegazzinii* by Downs, Aitken and Anderson (1955), this being the only species of the genus recognized by them on the island. The present report includes the first isolations of yellow fever virus from *Haemagogus mesodentatus*, *Sabethes chloropterus* and pure pools of *H. equinus*. In a

few instances, the mosquitoes contained recently ingested blood and the presence of virus may have been due to this factor.

Galindo, Rodaniche and Trapido (1956) in laboratory transmission experiments found that a high proportion of *H. mesodentatus*, together with its subspecies *gorgasi*, harbor virus and that this species readily transmits by bite. This finding, together with the frequent isolations from naturally infected specimens both during and after simian epizootic activity in Guatemala, clearly demonstrates the importance of this species as a natural vector.

The capacity of South American strains of *H. equinus* to transmit yellow fever virus by bite was originally demonstrated by Waddell and Taylor (1945). In the previously cited report, Galindo *et al.* (1956) showed that Central American strains also possess this capacity. The present work represents the first isolations of virus from pure pools of wild-caught *H. equinus*. However, Boshell and Osorno-Mesa (1944) isolated virus from a mixed pool of *Haemagogus* containing *equinus, spegazzinii falco* and *lucifer*.

Virus was isolated also for the first time from *Sabethes chloropterus*, although it had previously been recovered once by Shannon, Whitman and Franco (1938) from a mixed pool of sabethines including Sabethoides, Limatus, Wyeomyia and Trichoprosopon. One successful transmission by bite was obtained with colonized *Sabethes chloropterus* by Galindo and co-workers (1956), but virus was never recovered from specimens inoculated into mice. It is possible that there may be individual variations within the species to account for this discrepancy. At any rate, the status of *S. chloropterus* as a vector of yellow fever merits further investigation.

CONCLUSIONS

Isolation of yellow fever virus from naturally infected *Haemagogus mesodentatus, H. equinus* and *Sabethes chloropterus* captured in Guatemala is reported. The frequency of isolation from *H. mesodentatus* indicates that this species is epidemiologically the most important in that area of the three species mentioned.

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LEPTOSPIROSIS IN MALAYA

I. Sporadic Cases among Military and Civilian Personnel[1]

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The presence of leptospirosis in Malaya was noted originally by Fletcher (1928) who demonstrated infection in man, dogs and several species of rats. Subsequent observations have documented the continued occurrence of this disease in human beings (Danaraj, 1950; Mankikar, 1951) and recently its importance as a military problem in this country has been emphasized (Broom, 1953).

The high incidence of leptospiral antibodies in man, domestic animals and wild rodents (Wisseman *et al.*, 1955) suggested that infection by this group of organisms was widespread in Malaya.

During the course of an investigation of the etiology of short term fevers in Malaya it became apparent that leptospires were a frequent cause of febrile illnesses among foreign-military and indigenous-civilian personnel living in that country. The present report presents data bearing on the incidence of leptospirosis among patients hospitalized because of an acute febrile disease and describes some of the pertinent clinical and epidemiological features of this disease.

MATERIALS AND METHODS

Selection of patients. Patients included in the study of pyrexias of unknown origin (PUO) were adult males admitted to the British Military Hospital (BMH), Kinrara, between March, 1954 and September, 1955 or to the Institute for Medical Research (IMR) Ward, General Hospital, between July, 1954, and October, 1955. All patients with an acute febrile illness were included in the study except those with demonstrable malaria parasites in their blood at the time of admission to the hospital and those whose only symptoms were gastrointestinal or respiratory. Military patients admitted to BMH Kinrara were derived from units operating chiefly in the States of Pahang, Selangor and Negri

[1] This study was carried out under the sponsorship of the Commission on Immunization of The Armed Forces Epidemiological Board and was supported in part by a contract between The Office of the Surgeon General, Department of the Army and The Institute for Medical Research, Kuala Lumpur, Federation of Malaya.

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Sembilan. Civilian patients, indigenes of Malay, or of Chinese or Indian racial origin, who resided in or near Kuala Lumpur were admitted to the IMR Ward.

Clinical and laboratory observations compatible with the acceptable care of patients in a general hospital included physical examination, total and differential leukocyte counts, examination of blood films for malaria parasites and urinalyses. In selected patients, blood urea determinations, liver function tests and roentgen examinations of the chest were performed.

Intravenous alimentation was employed in the few patients who were unable to take fluids by mouth; otherwise liquid or soft diets were given during the acute phase of the illness. Special attention was paid to fluid balance in patients with renal decompensation. Some of the military patients suspected of having leptospirosis at the time of admission were treated with chloramphenicol or penicillin administered in daily amounts of 4 grams and 2.4 million units respectively (Fairburn and Semple, 1956). A few of the civilian patients received 3 grams of terramycin daily as soon as the clinical diagnosis of leptospirosis had been made.

Special laboratory studies. An attempt was made to establish a specific etiologic diagnosis in every patient admitted to the study, either by the isolation of an infectious agent during the illness or the demonstration of the appearance of specific antibodies in convalescence. Since many of the disease entities were not clinically distinct, certain procedures were performed routinely, providing the patient's illness satisfied certain criteria. Thus, specimens of whole blood or plasma, when obtained during the first 72 hours of illness, were inoculated intracerebrally into suckling-mice in attempts to recover viral agents. Such materials obtained during, but not after, the febrile period were cultured for leptospires and other organisms. Blood for serologic tests was drawn from most patients during the acute phase of the illness and again during convalescence. Frequently, in illnesses of very short duration, patients were not available for bleeding after the 15th day of disease; in many other cases, however, additional serum specimens were obtained during the 4th or 5th week. All sera were stored at $-20°C.$ prior to and after testing. Of the special laboratory procedures employed in this investigation, only those applicable to the diagnosis of leptospiral infection will be described in detail.

A modification of the cultural method described by Gochenour and his associates (1953) was employed for the isolation of leptospires from human blood. Freshly drawn venous blood was inoculated in 0.02 ml. amounts into 2 ml. of Fletcher's semisolid medium (Fletcher, 1928) which had been layered onto 5 ml. of nitrate agar (Difco) in a screw-cap tube. In most instances only one attempt was made to isolate leptospires from each patient and an average of three tubes was employed for each attempt. Inoculated tubes were held at room temperature (28–35°C.) in the dark for 30 days. Initially, cultures were examined by darkfield illumination on the 7th, 14th, 21st and 30th days for the presence of leptospires. After it was found that few cultures were visibly positive within the first week of incubation, darkfield examination on the 7th day was omitted. Aliquots of fluids containing leptospires were subcultured into Fletcher's medium

in glass ampules which were sealed and forwarded to a reference laboratory for definitive identification[4].

Mice which developed signs of encephalitis following the inoculation of blood were examined for leptospires. Passage of brain material to other mice was deferred when leptospires could be demonstrated in saline extracts of brain. Whenever the cause of encephalitis was in question, brains were passaged to other mice or stored at −70°C. until the results of blood cultures were known.

Serodiagnosis of leptospirosis in Malaya was achieved by the use of complement-fixing antigens prepared from sonically vibrated *Leptospira icterohemorrhagiae, L. grippotyphosa* and *L. hyos* (Randall *et al.*, 1949) and supplied by the Division of Veterinary Medicine of the Walter Reed Army Institute of Research (WRAIR), Washington, D. C. The three antigens were rehydrated, pooled in equal parts and diluted with 0.85 per cent sodium chloride in glass distilled water to a final dilution of 1:5 for each antigen. When the diagnosis was not established in a given patient by isolating leptospires from his blood, convalescent serum was screened by testing 1:5, 1:10 and 1:20 dilutions of serum with this trivalent antigen. Three dilutions of serum were employed in the screen test because some positive sera gave a prozone reaction. Titration of antibodies was then performed on paired sera from the patients on whom positive results had been obtained in the screen test and on paired sera from those patients whose illness was initially identified by the isolation of leptospires. This procedure employed 0.25 ml. of serial twofold dilutions (1:5 through 1:640) of acute and convalescent serum which had been inactivated for 30 minutes at 56°C. To this was added two full units of complement in 0.5 ml. amounts and 0.25 ml. of the trivalent leptospiral antigen. Overnight incubation at 4°C. was followed by the addition of 0.5 ml. of sensitized cells containing equal parts of 3 per cent sheep erythrocytes and 3 units of amboceptor. Complement-fixation was read after incubation at 37°C. for 30 minutes. The serum titer was taken as the highest dilution of serum giving 3+ or greater fixation of complement under these conditions.

Serum specimens from all patients whose illness was diagnosed as leptospirosis were forwarded to the Division of Veterinary Medicine, WRAIR, for detailed study by agglutination-lysis. In addition, sera from 70 patients whose illness, although clinically resembling leptospirosis, was not identified by cultural or complement-fixation procedures were tested by agglutination-lysis. This particular series of tests, performed with 14 serotype strains of leptospires selected on the basis of their prevalence in Malaya as well as their spectrum of cross reactions (Alexander *et al.*, 1955), yielded a diagnosis of leptospirosis in 16 cases which have been included in the present study.

In this study, a patient was diagnosed as having leptospirosis if one or more of the following criteria were satisfied:

1. Isolation of leptospires from the blood.

[4] Facilities for the identification of leptospiral isolates were provided by the Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D. C. and by Dr. J. C. Broom of the Wellcome Laboratories of Tropical Medicine, London.

2. Fourfold or greater rise in complement-fixing or agglutination-lysis antibody titer, or both, during the course of the illness.
3. Sustained titer of 1:40 or greater by complement-fixation or 1:400 or greater by agglutination-lysis when the first serum specimen was obtained on or after the 7th day of illness.

Only 9 cases of leptospirosis were included in the present series on the basis of the 3rd criterion alone.

RESULTS

Incidence of leptospirosis among military and civilian patients. During the course of this investigation, 852 patients with an acute febrile illness were studied. In this series of 614 military personnel and 238 civilians, leptospirosis was a major cause of febrile disease and accounted for 35 per cent of the illnesses in the former group and 13 per cent in the latter (Table 1). Scrub typhus, malaria and a group of diseases serologically related to dengue accounted for most of the remaining illnesses which were identified. The etiology of approximately 30 per cent of these fevers has remained unknown but paired sera from the patients with PUO have been maintained frozen and will be examined for antibodies against other agents.

Clinical features. Detailed observations on the clinical manifestations of leptospirosis were made in 209 of the 244 patients with this disease. Comparison of the patients from whom leptospires were cultured with those on whom the diagnosis was established solely by serologic means revealed no conspicuous differences in the clinical features of the illness. The few differences which were observed between military personnel and non-European civilians will be discussed below.

Leptospirosis in Malaya was an acute, self-limiting, febrile disease of about 8

TABLE 1

Identification of febrile diseases in military and civilian personnel in Malaya, 1954–1955

Disease category	Military patients		Civilian patients	
	Number	Percentage	Number	Percentage
Leptospirosis	213	34.7	31	13.0
Undiagnosed pyrexia (PUO)	176	28.7	73	30.6
Scrub typhus	69	11.3	7	2.8
Malaria	58	9.4	18	7.4
Dengue	42	6.8	56	24.0
Infectious mononucleosis	10	1.6	0	0
Murine typhus	4	0.6	8	3.4
Typhoid fever and other salmonelloses	3	0.5	12	5.0
Influenza	0	0	5*	2.1
Miscellaneous	39	6.4	28	11.7
Total	614	100.0	238	100.0

* Three cases were caused by A and two by B influenza virus.

TABLE 2

Clinical manifestations of leptospirosis

Symptom—Sign	Military patients			Civilian patients		
	Total recorded	Number positive	Per cent	Total recorded	Number positive	Per cent
Headache	172	164	95.3	28	26	93
Gastrointestinal symptoms	168	161	95.8	28	27	96.4
Conjunctival injection	169	144	85.2	28	23	82.1
Myalgia	173	134	77.4	28	16	57.1
Respiratory symptoms	165	117	70.9	29	25	86.2
Abrupt onset	158	111	70.2	24	24	100
Retrobulbar pain	154	85	55.1	27	11	40.8
Lymphadenopathy	166	74	44.5	29	15	51.7
Muscle tenderness	51	22	43	24	16	66.7
Abdominal tenderness	59	25	42.3	24	13	54.1
Nuchal rigidity	163	59	36.2	30	11	36.6
Photophobia	153	47	30.7	27	7	25.9
Joint pain	155	45	29	28	18	64.2
Hemorrhagic signs	160	44	27.5	29	7	24.1
Rash	164	44	26.8	28	5	17.8
Splenomegaly	162	26	16	29	5	17.2
Hepatomegaly	160	22	13.7	30	5	16.6
Jaundice	150	6	3.8	27	5	18.4

days' duration with extremes of 4 to 13 days. While the disease was usually not attended by a high mortality, fatal cases did occur and were observed twice (0.8 per cent) in this series. Although there was marked variation in the severity of symptoms from one case to another, the general clinical syndrome was repeated with remarkable consistency. The onset of symptoms was usually abrupt and the syndrome characterized by headache, conjunctival injection, retrobulbar pain, generalized myalgia, malaise and gastrointestinal disturbances. A detailed tabulation of clinical manifestations is given in Table 2.

The illness began with rigor in about half of these patients. Myalgia, when present, was most severe in the lower extremities and in abdominal and pectoral muscles. Backache and pain in the neck frequently were severe. Although civilian patients did not often complain of myalgia, joint pain and muscle tenderness frequently were noted. In contrast, less than half of the military patients had muscle tenderness and in few instances could it be called exquisite. This difference may have been due in part to difficulties in establishing good verbal rapport with the civilians. In both groups, headache was universally present and was accompanied by retrobulbar pain in 50 per cent of the patients. Some degree of conjunctival injection was noted in about 85 per cent of the group, however, photophobia was present in less than one-third. Rash which was observed in about 25 per cent of the patients appeared between the 4th and 8th day of disease and varied in duration from a few hours to several days. The eruption consisted of small macules or maculopapules and was distributed over the head and neck, trunk and upper extremities. In some patients, the lesions

were not unlike the rose spots of typhoid fever. One patient developed an urticarial eruption which persisted for several days.

Gastrointestinal disturbances consisting of anorexia, nausea, vomiting or abdominal pain occurred in 95 per cent of these patients. Protracted vomiting was the most distressing feature of the illness in many of the European patients. In this group, abdominal pain as a rule was generalized and mild, but on one occasion it was severe enough to provoke the removal of an appendix. In contrast, Asian patients seldom had severe vomiting, but complained more frequently of marked abdominal pain.

Some form of respiratory complaint was noted in 70 per cent of the military and 85 per cent of the civilian patients. Cough was the most common single respiratory abnormality and was productive of bloody sputum in 25 per cent of individuals with this symptom. Coryza and sore throat were not prominent features of the disease. Roentgen examination of the chest was performed in 42 patients, 11 of whom exhibited some abnormality. Five of the patients with x-ray changes had clinical evidence of pneumonitis and 4 had hemoptysis. X-ray findings varied from slight increases in vascularity to diffuse infiltration with small localized areas of increased density. One individual had lobar pneumonia with clinical signs of consolidation. Leptospires were isolated from 7 of the 11 patients with roentgen evidence of pneumonitis and among these were members of the schüffneri, grippotyphosa, australis B, canicola and pyrogenes serogroups.

Hemorrhagic phenomena were not common and in only two instances was bleeding a serious problem. Both patients experienced massive hemoptysis and one ultimately succumbed to an illness caused by *L. australis B*. When present, hemorrhagic signs added considerable weight to the diagnosis of leptospirosis as they were rarely observed in other diseases. Hemoptysis was the most common form of bleeding followed by hematemesis, conjunctival hemorrhage, epistaxis, petechiae and melena.

Although jaundice was more common in Asian patients than Europeans, it was not a prominent part of leptospiral infection in either group. Leptospires of the following serogroups were isolated from 8 of the 14 patients with clinically overt jaundice: icterohemorrhagiae, 2; schüffneri, 1; hebdomadis, 1; pyrogenes, 1; bataviae, 1; canicola, 2. The infrequent occurrence of splenomegaly (16 per cent) was conspicuous and constituted a differential diagnostic criterion of some value since it occurs with great frequency in two other common febrile illnesses in Malaya, scrub typhus and typhoid fever.

Certain features of the disease were difficult to assess and for this reason no attempt was made to use them as differential diagnostic points. Generalized adenopathy was noted in about 45 per cent of all patients, but the degree of glandular enlargement did not approach that of scrub typhus or dengue. Nuchal rigidity was present in about 36 per cent of the entire group, however, as few lumbar punctures were performed, the incidence of meningeal involvement is not known. Cerebrospinal fluid was shown to contain increased cells (40–400/cmm.) and protein during the first week of illness in 6 of the 10 patients on whom this examination was performed.

Recovery from leptospiral infection was rapid and in most instances defervescence was attended by the disappearance of all symptoms. Antibiotic therapy with chloramphenicol, penicillin or terramycin did not appear to alter the course of disease in this series (Fairburn and Semple, 1956). Iridocyclitis and other ocular complications were not observed in this group of patients. Convalescence was usually uneventful and most individuals were able to return to their daily routine within three weeks after the onset of symptoms. Military patients who became ill from any cause within the succeeding months were observed carefully for evidence of leptospirosis. One patient who suffered two attacks of leptospirosis within a three month period is of particular interest. This individual first became ill in April, 1954, with fever, conjunctival injection, myalgia, vomiting, urinary abnormalities, lethargy and nuchal rigidity. Lumbar puncture revealed normal cerebrospinal fluid, but lethargy continued for several days. The illness subsided after 8 days. Blood culture during this episode yielded a leptospire subsequently identified as *L. schüffneri*. The patient was asymptomatic until July, 1954, when he again experienced fever, myalgia, conjunctival injection, vomiting and hemoptysis. This illness ended in 4 days and blood culture at this time revealed the presence of a leptospire later shown to be *L. paidjan*.

Clinical laboratory studies. Examination of the total leukocyte counts revealed that most patients with leptospirosis had a normal number of white blood cells, however, both leukocytosis and leukopenia did occur. Analysis of the differential counts showed that neutrophilia was usually the rule regardless of the total number of leukocytes. Thus, 80 per cent of patients on whom differential leukocyte counts were made showed 70 per cent or more neutrophils during the febrile phase of the illness (Table 3). This observation is considered to be of particular value in Malaya where typhus fevers, typhoid and dengue, in which there is a distinct tendency toward neutropenia, must be differentiated from leptospirosis.

During the first week of disease, approximately 80 per cent of the military

TABLE 3

Leukocyte patterns in leptospirosis

Leukocyte count	Military patients		Civilian patients	
	Number	Percentage	Number	Percentage
Total leukocytes				
Normal	100	61.3	13	44.8
Leukocytosis	50	30.6	14	48.2
Leukopenia	13	8.1	2	7.0
Total	163	100.0	29	100.0
Differential				
PMN 70% or more	63	81.8	24	80
PMN 69% or less	14	18.2	6	20
Total	77	100.0	30	100.0

TABLE 4

Biochemical studies in patients with leptospirosis

Test	Criterion of abnormality	No. tests performed	No. patients tested	Patients with abnormal findings	
				Numbers	Percentage
Bilirubin	1.0 mg./100 ml.	100	49	26	53
Alkaline phosphatase	13 King-Armstrong units	56	16	10	62
Kunkel's test	10 Kunkel units	109	36	27	75
A/G ratio	1.1	64	38	24	63
Blood urea	40 mg./100 ml.	132	60	30	50

patients exhibited some urinary abnormality as manifested by the appearance of proteinuria, cylindruria and an increase in cellular elements. The higher incidence of abnormal urine in the group with demonstrated leptospiremia (87 per cent) as compared with the group in whom cultures were negative (69 per cent) provided the only evidence to suggest that a more severe form of the disease was encountered in those patients whose blood yielded a leptospire. In most instances, albuminuria was not intense, indeed, it might have been attributed solely to the febrile state by some observers. Similarly, in some cases there were small numbers of casts and cellular elements. Signs of renal derangement as measured by urinalysis usually disappeared rapidly after the 7th day of disease.

Biochemical studies. Several biochemical tests designed to detect evidence of hepatic and renal dysfunction were performed on a group of patients with leptospirosis. Details of this phase of the study are presented in Table 4, which reveals that 50 to 75 per cent of these individuals had an abnormal liver function test at least once during the illness. In most instances, hepatic derangement, as measured by these tests, was mild and of short duration. Serum bilirubin concentration was elevated sufficiently to produce clinically overt jaundice in only 14 patients. Two of these patients succumbed during the first week of an illness caused by *L. bataviae* and *L. australis B* respectively.

As indicated previously, most patients produced abnormal urine during the acute phase of the illness. The appearance of proteinuria, cylindruria and in some instances oliguria was attended by some degree of elevation of blood urea in about half of the patients on whom this test was performed. The degree of renal decompensation usually was mild. Significant oliguria or anuria was rarely observed and, when present, did not persist longer than 48 hours.

Whenever evidence of hepatic or renal dysfunction did occur, it appeared during the febrile phase of the illness and rapidly disappeared during the second week of disease. The relationship of this dysfunction to phase of illness is presented in Figure 1.

Diagnostic laboratory studies. A diagnosis of leptospirosis was made in 228 patients on the basis of the criteria outlined earlier. These included demonstration of leptospiremia or of a fourfold increase in complement-fixing or agglutination-lysis antibodies in 219 cases. In 9 cases the laboratory evidence was limited to a persistent high level of complement-fixing antibodies. The identity of the illness

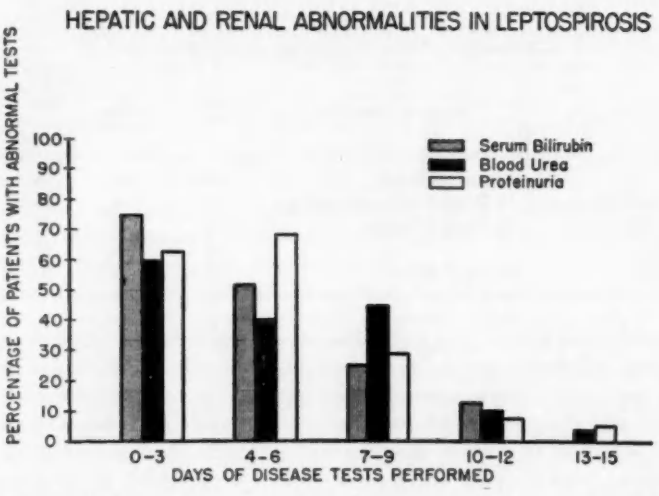

FIG. 1. Hepatic and renal abnormalities during the course of leptospiral infection

was established by agglutination-lysis tests in 16 patients in whom cultural and complement-fixation studies were negative.

Blood specimens from 162 military and 20 civilian patients in the group with leptospirosis were inoculated into Fletcher's medium on one or more occasions. No attempt was made to recover leptospires from the blood of 62 patients, half of whom were admitted to the hospital after the acute phase of their illness had passed. Leptospires were recovered from 108 of the 182 patients (59 per cent) to whom this diagnostic procedure was applied. During 1954, among military patients alone, when each blood sample was inoculated into 2 tubes of medium, 58 per cent of these cultural attempts were positive. In 1955, when 3 or more tubes were used in each isolation attempt and in some instances serial cultures were performed on selected patients, the incidence of positive cultures was 70 per cent. In 35 of 84 individual cultures which were positive, not all of the inoculated tubes contained leptospires after 30 days' incubation, indeed, in one instance, leptospires were present in only 1 of 8 tubes. Such observations suggest that organisms may be recovered when their concentration in the blood is of a low order. The relationship between phase of illness and recovery of leptospires is illustrated in Figure 2 indicating that positive cultures were obtained more frequently early in the febrile period which is in accord with the findings of others (van Thiel, 1948; Gochenour, 1953).

Eighty-two cultures which eventually proved to be positive, were examined by darkfield at weekly intervals. After 7 to 10 days of incubation, only 17 per cent of the cultures were positive but by the 21st day, 74 per cent contained leptospires. The remaining 25 per cent became positive during the fourth week of incubation. These observations are in accord with those of earlier workers (Wolff, 1954; Gochenour, 1953).

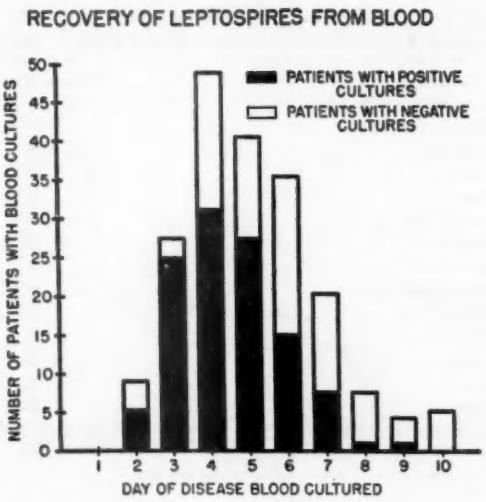

FIG. 2. Occurrence of leptospiremia during the acute phase of disease

The serologic characterization of leptospires from Malaya has been completed and will be the subject of another publication (Alexander *et al.*, to be published). Preliminary results, however, have disclosed the presence of 27 distinct serotypes which are distributed among 13 serogroups. It may be noted that 20 of these serotypes have not been isolated previously from man or animals in Malaya; in addition, the antigenic characteristics of 15 serotypes have hitherto not been recognized. Ninety of the 108 isolates obtained from patients in the present series have been identified and their distribution by serogroups is shown in Table 5.

During the course of the studies on pyrexias of unknown origin, acute-phase blood from 33 patients whose illness was shown subsequently to have been leptospirosis, was inoculated intracerebrally into suckling-mice. Fifteen of these specimens produced encephalitis and death in the inoculated animals after an incubation period of 5 to 10 days. Pathogenicity for suckling-mice was frequently lost on passage and leptospires did not produce overt disease in adult mice inoculated by the intracerebral route. Careful darkfield examination of suspensions of brain tissue of the affected mice usually revealed the presence of leptospires, however, in some cases identification of this organism among tissue debris was difficult. As the diagnosis of leptospirosis was easily accomplished by other means, little effort was made to subculture leptospires from brain material. However, in the few instances where this procedure was attempted, the organism was recovered. Comparison of isolation-rates in suckling-mice with those in Fletcher's medium revealed that the latter method was twice as effective.

Complement-fixation tests were performed on serial specimens of serum from 210 patients with leptospirosis, 190 (90 per cent) of whom developed comple-

TABLE 5

Serogrouping of leptospires of human origin in Malaya, 1954–1955

Serogroup	Number of strains
pyrogenes	18
hebdomadis	10
bataviae	10
canicola	9
grippotyphosa	8
autumnalis	8
celledoni	8
icterohemorrhagiae	6
schüffneri	5
australis A	4
javanica	2
pomona	1
djasiman	1
Total	90

ment-fixing antibody during the course of the illness. In all instances, a fourfold or greater increase in such antibody was demonstrated when the first serum specimen in the series was obtained prior to the 7th day of disease. Contrariwise, a rise in titer of complement-fixing antibody was not demonstrated in 9 patients who came under observation after the first week of illness. Complement-fixing antibody usually made its appearance during the second week of illness and rapidly reached maximum titer. Those patients destined to develop complement-fixing antibody did so by the 15th day of disease in all instances. The geometric mean maximum titer reached by 75 patients was 1:107 with extremes of 1:10 and 1:640. Complement-fixation tests for leptospirosis performed on 160 patients with PUO yielded negative results in all instances.

Agglutination-lysis antibodies against one or more serotypes appeared or increased in titer during convalescence in the sera of all patients from whom leptospires were recovered, or who developed complement-fixing antibodies. Sixteen other patients developed agglutination-lysis antibodies during a febrile illness which could not be identified by cultural or complement-fixation procedures. These 16 persons were among a selected group of 70 whose illness resembled leptospirosis. More extensive use of the agglutination-lysis test on sera from the remaining 195 patients classified as PUO might have revealed additional cases of leptospirosis, however, it is unlikely that the incidence would be as great as that observed in the specially selected group.

Epidemiologic observations. Study of the habits of patients with leptospirosis during 1954 and 1955 provided additional support for the concept that this disease is contracted in a variety of environments in Malaya. It was apparent from earlier observations that exposure to leptospires could occur in urban and cultivated-rural areas as well as scrub and primary forest (Fletcher, 1928; Broom, 1953; Wisseman, 1955). In the present study, the frequent appearance of this

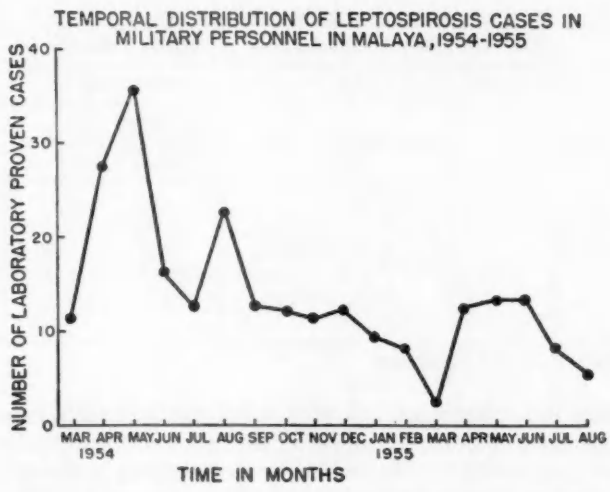
TEMPORAL DISTRIBUTION OF LEPTOSPIROSIS CASES IN MILITARY PERSONNEL IN MALAYA, 1954-1955
NUMBER OF LABORATORY PROVEN CASES
0
10
20
30
40
MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG
1954
1955
TIME IN MONTHS

FIG. 3. Temporal distribution of leptospirosis cases in military personnel in Malaya, 1954–1955.

disease among men whose occupation took them into jungle suggests that this particular environment represents one of the most important natural foci of leptospiral infection in Malaya.

Table 1 shows that the incidence of leptospirosis among military and civilian patients in this study was high but that this disease was more common in the former group. Differences in the attack rates in the two groups were affected by numerous factors including opportunity for exposure and possible presence of previously acquired immunity. For this reason, military patients will receive particular attention in comparative analyses of epidemiologic factors.

The temporal distribution of all cases of leptospirosis diagnosed in the current study indicates that the disease may be acquired in any month of the year in Malaya. The distribution of cases in military personnel (Figure 3) reveals a concentration of cases in the months of April and May, 1954. This was associated with the recent arrival of two units in a hyperendemic area and is probably not a reflection of seasonal incidence. Comparison of number of military cases per month with rainfall figures for 1954 and 1955 showed no correlation with disease and overall or localized precipitation.

Racial distribution of cases in military and civilian groups is presented in Table 6, where the incidence in the former group is expressed as attack rates per 1,000 troops at risk in jungle and rural areas per annum and in the latter group as percentage of total cases studied. The overall figures suggest that soldiers of European origin may have been more susceptible to infection than military personnel of other races. However, more detailed analysis of the attack rates in European troops showed a marked disproportion of cases from Western Pahang

TABLE 6

Racial distribution of leptospirosis in Malaya 1954–1955

Military patients		Civilian patients			
Race	Attack rate*	Race	Percentage distribution within study group	No. cases	Per cent cases
European					
Western Pahang	47	Indian	48.9	19	61.3
Other Areas	9	Malay	25.3	8	26.0
Gurkha	4	Chinese	21.6	3	9.7
African	3	Other	4.2	1	3.0
Other	2				

* Per 1,000 men at risk per annum

in comparison with other areas. This high attack rate in Western Pahang points to geographic areas of hyperendemicity as the important factor rather than increased racial susceptibility. The incidence of leptospirosis in the different non-European races among civilian patients was about the same as the proportion of the several races admitted to the study group. The slightly higher rate of infection in Indians and correspondingly lower rate in Chinese, than would be anticipated on the basis of distribution of races within the study, may have been influenced to some extent by the habits and occupations of the two races; many of the Indians lived or worked in rural areas while most of the Chinese were urban dwellers. Of the 28 non-European patients whose histories contained adequate epidemiologic data, 16 probably were infected in rural areas and 7 in the city of Kuala Lumpur. The remaining 5 persons gave urban addresses but had either worked in or visited a rural area during the 3 weeks prior to the onset of symptoms. Among the 16 rural dwellers, 4 were employed on rubber estates and 3 in tin mines.

Sufficiently detailed information was available to determine with considerable assurance the area in which 192 soldiers contracted leptospirosis. The study area including Perak, Pahang, Selangor and Negri Sembilan is outlined in Figure 4. Other data clearly indicated that military cases occurred in two other states (Johore and Kedah), however, the fragmentary nature of the observations is inadequate to warrant a statement on the prevalence of leptospirosis in these two areas as well as in the States of Trengganu, Kelantan and Perlis.

Particular attention should be given to the large number of cases in Western Pahang where several European units operated for long periods of time. Reference to Table 6 shows that the attack rate among Europeans in this area was 47 per 1,000 men at risk per annum. This high attack rate was not approached in other areas where either Caucasians or troops of other races were operating. The overall attack rates expressed on the basis of 1,000 troops of all races at risk per-annum were 55, 18, 12 and 6 for Pahang, Perak, Selangor and Negri Sembilan, respectively.

Additional evidence that Western Pahang was a zone of high endemicity was

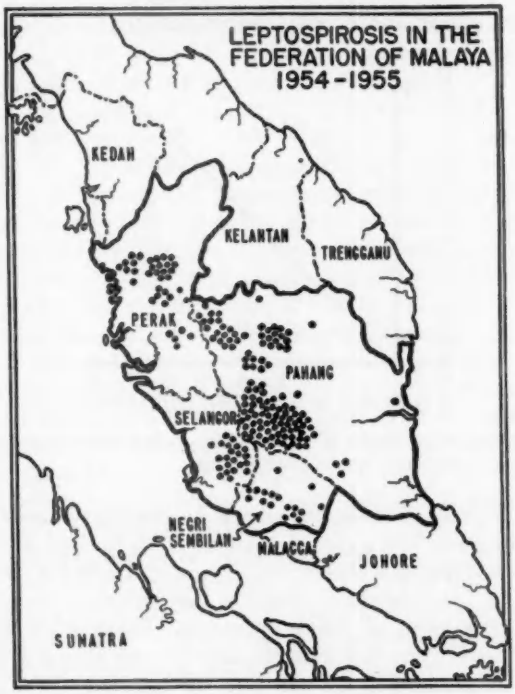

FIG. 4. Geographical distribution of cases of leptospirosis in Malaya

TABLE 7

Occurrence of leptospirosis in units operating in identical areas at different times

Unit	Year	Operational area	Case rate per unit time
A	1954	Pahang	63.0
	1955	Selangor	3.7
B	1954	Selangor	7.2
	1955	Pahang	30.0

provided by the records of two military organizations of equal size which performed similar operations alternately in Western Pahang and Selangor. The data presented in Table 7 show that the case rates per unit of time were markedly higher while each organization was located in Pahang. Somewhat similar observations were made on another unit of 300 men which operated successively in Perak, Western Pahang and Negri Sembilan where the case rates per unit of time were 1.5, 6.0 and 0, respectively.

Leptospirosis was nearly 5 times as common in units operating in jungle as in those units whose activities were primarily of a supportive nature. Furthermore,

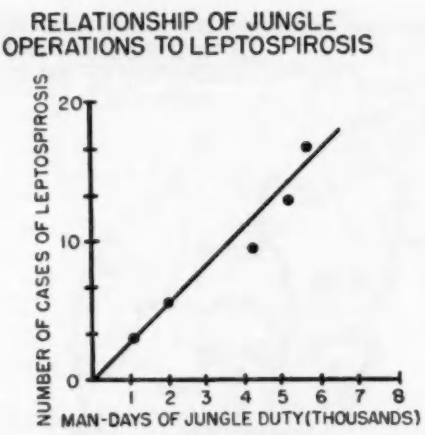

FIG. 5. Comparison of incidence of leptospirosis in five military groups of equal size operating in same area during a period of 11 months.

an appreciable number of infections among nonoperational personnel involved persons who had performed a special mission within forested areas prior to the illness. The concept that this disease usually was contracted in the jungle is supported further by the observation that the case incidence was proportional to the amount of time spent on jungle operations (Figure 5). Thus, the highest attack rate occurred in the unit with the heaviest operational schedule and the lowest attack rate occurred in the unit with the least amount of jungle duty.

Within those units engaged in jungle operation, only 10 per cent of the patients with leptospirosis denied having been in jungle during the 19 days prior to onset of symptoms. The exact dates and areas of operation were established with certainty in 80 patients. Seven of these persons had been in the jungle continually during the incubation period of 4 to 19 days defined by Schüffner (1934). Fifty-two of the remaining 73 patients had been in jungle for over half of the incubation period and of these, 35 had spent two-thirds of this time on jungle operation.

Distribution of cases by rank was of the same order as the distribution of ranks within a unit except among officers. The occurrence of twice as many cases among the officers as would be anticipated on the basis of their numbers is probably a reflection of their additional contribution to the total operational effort.

DISCUSSION

Leptospirosis has been recognized as a disease of great importance in Indonesia for many years. Monumental studies of the clinical and epidemiologic features of this malady in the Netherlands Indies carried out by numerous workers have been summarized by Walch-Sorgdrager (1939) and Collier (1948). With the notable exception of Fletcher's work in Malaya (1928), comparable investigations on leptospirosis in other countries of Southeast Asia had not been undertaken until recently when war in Viet-Nam and the anti-terrorist operations in Malaya

focused attention on this disease (Broom, 1953; de Lajudie and Brygoo, 1953). The present study provides additional information on the prevalence of leptospirosis in Malaya. All of these investigations suggest that leptospires are a common cause of human disease throughout Southeast Asia.

The clinical manifestations of leptospiral infection in Malaya do not differ appreciably from those described by other workers in this and other parts of the world (Schüffner, 1934; Walch-Sorgdrager, 1939; van Thiel, 1948; Gsell, 1952; Woodward, 1953). In Malaya, any illness characterized by headache, myalgia, conjunctival injection and gastrointestinal symptoms in the presence of urinary abnormalities warrants the diagnosis of leptospirosis until proven otherwise.

The low incidence of clinically overt jaundice and death in the present series of cases differs somewhat from the experience of workers in Europe, North America and other parts of Asia (Schüffner, 1934; Walch-Sorgdrager, 1939; van Thiel, 1948; Broom and Alston, 1948; Hall 1953) but is in general agreement with the studies in Indonesia reviewed by Walch-Sorgdrager (1939). Many factors probably contribute to the relative mildness of leptospirosis in Southeast Asia. First, under the conditions of the present investigation, mild infections were identified which might not have been recognized without the use of intensive laboratory studies applied to the entire group of patients with febrile disease. Second, *L. icterohemorrhagiae*, *L. bataviae*, *L. andaman A* and *L. australis A* which have been associated with a high incidence of icterus elsewhere were either absent from this group or caused few cases. Furthermore, it should be noted that the clinical manifestations associated with infection by a single serotype may vary from one geographical area to another as in the case of *L. bataviae* (Austoni, 1953; Walch-Sorgdrager, 1939). Leptospires of the serogroups, pyrogenes, hebdomadis, canicola and grippotyphosa, which are accepted as usually producing a relatively mild disease in man, accounted for 50 per cent of illnesses in patients in this series from whom leptospires were isolated. The symptoms produced in man by some of the other serotypes (*L. schüffneri*, *L. javanica*, *L. djasimani* and new serotypes) have been described rarely, if at all, but it would appear from this study that they, too, cause a mild form of infection. Finally, it should be emphasized that classifying infection on the basis of the presence or absence of clinically overt jaundice is somewhat academic in view of the finding of biochemical evidence of hepatic dysfunction in 50 per cent of patients in the present series studied in this manner. It is of some interest that the incidence of icterus was higher in the civilian group than in the military. Whether this reflects the fact that soldiers are more apt to be hospitalized with any mild illness than are civilians or whether it suggests that the young, healthy, well-nourished troops are prone to have a mild disease cannot be stated.

Results obtained with the complement-fixation test in Malaya were comparable to those of Gochenour in Puerto Rico (1953) and indicated that sonic-vibrated antigens may have a world-wide application in the diagnosis of leptospiral infections of man. Complement-fixation tests confirmed the diagnosis of leptospirosis in 90 per cent of patients in the present series and the appearance of these antibodies during the second week of disease was helpful in establishing

an early diagnosis. The broad specificity of the lyophilized sonic-vibrated trivalent leptospiral antigens was another desirable diagnostic feature of the complement-fixation technique. Despite these advantages, the classical cultural procedures and the type specific agglutination-lysis technique continued to retain important places in the diagnosis of leptospirosis.

It should be pointed out that circumstances coincident to the anticommunist activities in Malaya have provided a unique opportunity to observe comparable groups of people in a variety of environments some of which are not ordinarily frequented by man. For this reason most of the epidemiologic investigations have been limited to those individuals engaged in this war because their activities could be traced with accuracy and their total number within each type of environment was known. For the most part, these patients were European military personnel, foreign to Malaya, and one can probably assume them to be non-immune. The results of this study have established that leptospirosis was encountered more commonly in mature forest than elsewhere and that within the forest there existed hyperendemic foci of leptospiral infection.

Leptospires probably gained access to the human host through the skin of the lower extremity since this portion of the body was most frequently exposed to streams, stagnant pools, marshy lowlands, pig wallows and other likely sources of contamination. The presence of cutaneous lesions produced by wet boots and leech bites undoubtedly increased the chance of infection. However, other routes of infection should not escape attention; for example, during jungle operations it was sometimes necessary to drink water from sources which were less than ideal.

The Malayan forest has a rich fauna and at least three species of rats which inhabit the primary jungle (*Rattus mulleri*, *R. rajah* and *R. sabanus*) have given evidence of infection with leptospires (Wisseman, 1955). Thus, these rats and other animals may serve as important reservoirs of this micro-organism. The conditions under which hyperendemic foci are formed and maintained in the Malayan jungle provide a fertile field for future studies.

SUMMARY

A study of 852 patients with acute febrile illnesses revealed that leptospirosis was a common disease in Malaya, having been the cause of 35 per cent of fevers among foreign military personnel and 13 per cent among adult male non-European civilians. The diagnosis of leptospirosis was accomplished by a combination of procedures including cultural, complement-fixation and agglutination-lysis techniques. Under the conditions of this study, approximately 60 per cent of those patients whose blood was cultured had demonstrable leptospiremia, 90 per cent developed complement-fixing antibodies and all had demonstrable agglutination-lysis antibodies during convalescence.

Leptospirosis in this series of patients was characterized by headache, gastrointestinal disturbances, conjunctival injection and a febrile course of about 8 days' duration. Varying degrees of renal dysfunction as manifested by the excretion of albumin, cells and casts occurred in about 80 per cent of these pa-

tients. Clinical jaundice was uncommon but laboratory evidence of hepatic dysfunction was encountered in half the patients.

Patients acquired leptospirosis in a variety of environments in Malaya but contracted it most frequently in primary forest in which there appeared to be hyperendemic foci of infection.

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NEEDLE BIOPSY IN THE ETIOLOGIC DIAGNOSIS OF SPLENOMEGALY*

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This paper reports our experience with needle biopsy of the spleen in 55 patients. While liver biopsy has been well established as a diagnostic aid of undisputed value in the study of liver disease, percutaneous spleen biopsy or puncture is still regarded by many as a hazardous procedure of academic value only. The outstanding investigators in the field of spleen puncture have been Introzzi (1935), Pittaluga (1953), Emile-Weil and his co-workers (1936) and Moeschlin (1951). Successful biopsy with the Vim-Silverman needle was later reported upon by Block and Jacobson (1950), and Block (1953), who are of the opinion that sections are more informative than smears. Other publications in the English literature have been those of Hess (1930), Morrison *et al.* (1951), Chatterjea *et al.* (1952), and Ferris and Hargraves (1935).

The stimulus that initiated the present study was the inability to establish the endemic form of splenomegaly associated with schistosomiasis (Egyptian splenomegaly) by clinical examination alone and the danger of confusing it with other causes of splenic enlargement. The prevalence of this type of disease in Egypt has often tempted clinicians to include other types of splenic enlargement under the same heading. The examination of puncture material obtained by aspiration from the spleen in those with Egyptian splenomegaly does not help in the elucidation of the diagnosis or underlying pathologic change as reported by several authors (Day and Ferguson, 1909; Ibrahim, 1928; Onsy, 1935). Our work confirms this. The work of Block and Jacobson (1950) with needle biposy of the spleen suggested to us the possibility of employing this procedure to obtain a definite core of tissue from which the structural and architectural pattern could be studied in addition to the cytological changes, if any. In the light of our experience we have come to the conclusion that biopsy with the Vim-Silverman needle is no more dangerous than aspiration with a finer needle, contrary to the view of Moeschlin (1951) and Chatterjea *et al.* (1952). In the splenomegalies other than the endemic (Egyptian) type, however, where the different cells must be studied in great detail, we have combined the examination of sections from the needle biopsy with smear material from the same patient.

MATERIAL AND METHODS

Patients were selected from various hospitals on the basis of two criteria: 1) an enlarged spleen, and 2) no demonstrable bleeding tendency as determined by the

* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

platelet counts, and bleeding and coagulation times. Both the transthoracic route advocated by Moeschlin (1951) and the transabdominal route were utilized, though we prefer the latter. The needle biopsy material was fixed in neutral 10 per cent formalin in water and multiple sections were stained routinely with hematoxylin and eosin, Masson's trichrome, Gridley's reticulum, and Ziehl-Neelsen's acid fast stains. Other special stains were employed when it was considered necessary. For the smears made on slides the Wright-Giemsa stain was found highly satisfactory.

RESULTS

These are summarized in Table 1, together with the clinical diagnosis prior to obtaining the biopsy. The pathological findings in the cases of chronic congestive splenomegaly alone will be briefly described. Forty-four of our cases fall under this broad classification. They include all patients in whom the spleen showed evidence of increased portal pressure. The changes in the spleen following bilharzial fibrosis of the liver are, on the whole, not specific, and resemble those reported in other types of cirrhosis of the liver, Banti's syndrome, thrombosis of the splenic and portal veins, and others. They have been reported on at great length by numerous authors (Day and Ferguson, 1909; Day, 1925; Girges, 1934; Ibrahim, 1928; Onsy, 1935; Sorour, 1928). It is not the purpose of this paper to review or comment on these reports.

The changes noted were: thickening of the capsule, proliferation and thickening of the trabeculae, dilatation and congestion of the veins and sinuses, arteriolar thickening, hemorrhages around the vessels and the formation of Gamna-Gandy bodies, proliferation of the reticular framework of the spleen, and fibrosis of the pulp. These advanced changes were found in most of our cases. In some cases there was hyperplasia and active proliferation of the pulp cells. The follicles were atrophic and without germinal centers in all but one of the cases in which they were present. In addition to the normal spleen cells, plasma cells were noted in excess of normal, and in 14 cases eosinophils were noted in abundance. While we feel that the presence of eosinophils in the spleen is in favor of a schistosomal

TABLE 1

Results of 55 cases of spleen biopsy

Clinical diagnosis	Histological diagnosis	Number of cases
Egyptian hepato-splenomegaly	Chronic congestive splenomegaly	44
? Hodgkin's disease	Miliary tuberculosis	1
Splenomegaly of unknown origin	Gaucher's disease	2
Splenomegaly of unknown origin	Acute lymphatic leukemia	1
Chronic lymphatic leukemia	Chronic lymphatic leukemia	4
Chronic myeloid leukemia	Chronic myeloid leukemia	1
? Hodgkin's disease	Hodgkin's disease	1
Egyptian hepato-splenomegaly	Normal spleen	1
Total		55

TABLE 2

Correlation of pathology of spleen and liver in portal hypertension

Spleen biopsy	Liver biopsy	Number of cases
Chronic congestive splenomegaly (22 cases)	Bilharzial fibrosis	8 (36.5%)
	Bilharzial fibrosis (consistent with) Portal cirrhosis	6 (27%)
		8 (36.5%)
Total		22 (100%)

etiology, it must be borne in mind that other coexistent diseases may be the cause, and we believe that this does not provide sufficient evidence to separate the schistosomal from the non-schistosomal group of congestive splenomegalies. In a very few cases occasional megakaryocytes and erythroblasts were encountered pointing to extramedullary hemopoietic foci in the spleen. Abundant pigment granules (hemosiderin) were present in the fixed and wandering macrophages and lying free in the pulp reticulum.

On the basis of the above histological changes the diagnosis of chronic congestive splenomegaly was made to the exclusion of other causes of splenic enlargement. In 22 (50%) of these 44 patients with congestive splenomegaly, needle biopsy of the liver was also performed simultaneously to correlate the findings with those of the spleen. In every case the liver showed evidence of fibrosis. In 8 of these cases (36%) the liver fibrosis was associated with schistosomiasis; eggs or their remnants were detected in the sections. In another 6 cases (27%) the pathological changes were consistent with bilharzial fibrosis, though no eggs were found after repeated sectioning and with the use of the modified Ziehl-Neelsen stain advocated by Lichtenberg and Lindberg (1954). Eight patients (36%) with congestive splenomegaly showed the portal (or Laennec's) type of hepatic cirrhosis (see Table 2).

DISCUSSION

Needle biopsy of the spleen has, in our hands, proved a valuable and safe diagnostic procedure, no mortality or complication occurring in this series of 55 cases. Transient local pain was complained of by about a third of the patients. Most of the patients were observed in the hospital for at least 7 to 10 days following the biopsy, a number of them reporting later for follow-up visits.

We feel that the study of sections made from a core of tissue, combined with the examination of smears is the ideal method of studying the pathology of the spleen with a view to arriving at the correct etiologic diagnosis. Histological examination with routine and special stains will reveal many alterations which cannot be studied from smear material.

This paper has placed special emphasis on the value of needle biopsy in the study of the chronic congestive splenomegalies and in their differentiation from other causes of splenic enlargement. In the group of patients from whom liver

biopsies were also obtained, fibrosis or cirrhosis of that organ was noted in every case, whether of schistosomal origin or otherwise. A liver biopsy, however, may not be obtainable from all patients, especially those with advanced cirrhosis and shrinkage of the liver. In these cases the diagnosis of chronic congestive splenomegaly can be made by needle biopsy of the spleen. This will afford valuable information to the surgeon contemplating a shunt or other type of operation for the relief of portal hypertension. Needle biopsy of the liver and spleen should prove a valuable research weapon for following the stages of schistosomiasis in man, and the effect on these organs of the various therapeutic agents used in the treatment of that disease. The effect on the liver and spleen of various shunt operations and hepatic artery ligation used in the relief of the portal hypertension could also be studied by needle biopsy of these organs.

Our experience as regards the value of needle biopsy or puncture in other types of splenomegaly has been limited. In tropical and subtropical areas, puncture of the spleen has proved very useful to many workers in the diagnosis of such diseases as malaria, kala-azar, and trypanosomiasis. In the splenomegalies accompanying the lipoid storage diseases, spleen puncture may be necessary for a correct diagnosis. The aleukemic leukemias, psuedo-leukemias, and leukemoid reactions can best be differentiated by this procedure. In the leukemias serial biopsies are useful from a therapeutic and prognostic point of view (Moeschlin, 1951). Reticulum cell sarcoma, lymphosarcoma, lymphadenoma or other neoplasms confined to the spleen are best diagnosed by spleen biopsy. Myelosclerosis and myelofibrosis form a special group in which biopsy of the spleen is very important in order to avoid splenectomy, which may be fatal.

In conclusion we wish to reiterate that spleen biopsy appears to carry with it no greater risk than liver biopsy. It should be utilized in selected cases in conjunction with peripheral blood and bone marrow examinations when the etiological nature of enlargement of the spleen is unsettled.

SUMMARY

Needle biopsy of the spleen was performed in 55 cases of splenomegaly in Egypt to establish a diagnosis impossible by clinical examination alone. Cores of tissue thus obtained were sectioned and stained, providing a basis for differentiating chronic congestive splenomegaly associated with schistosomiasis from other etiologic types, which is hardly possible with aspirated material. The pathological changes in 44 cases of congestive splenomegaly are described, and correlated in 22 cases with needle biopsy material from the liver. Needle biopsy may be indicated in other types of splenomegaly, since the procedure proved to be without mortality or complications in this series of cases.

ACKNOWLEDGEMENT

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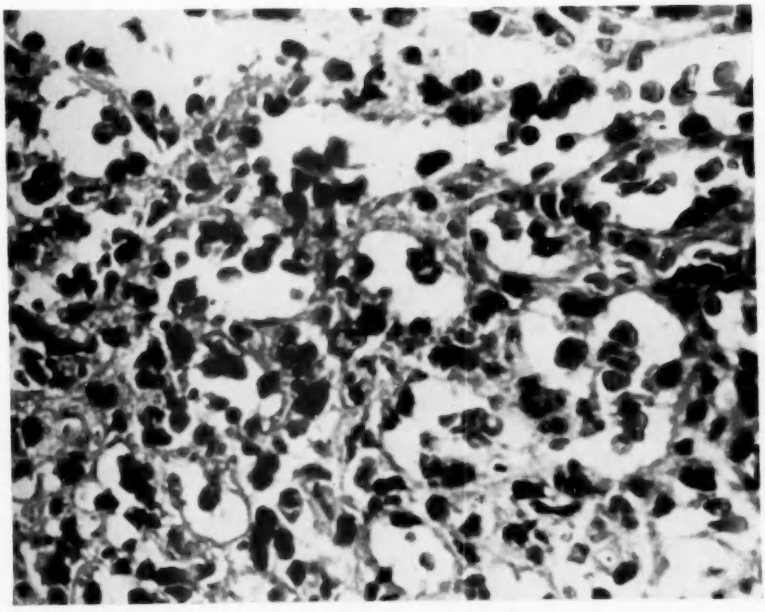

FIG. 1. Section of needle biopsy of spleen showing dilatation of sinuses in chronic congestive splenomegaly.

FIG. 2. Gamna-Gandy body around arteriole in spleen in chronic congestive splenomegaly.

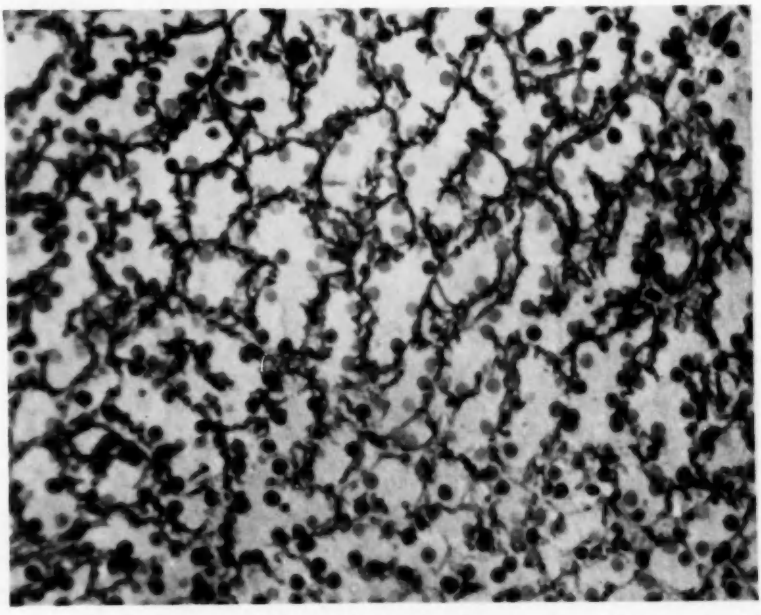

FIG. 3. Marked increase in reticular framework of spleen in Egyptian splenomegaly.

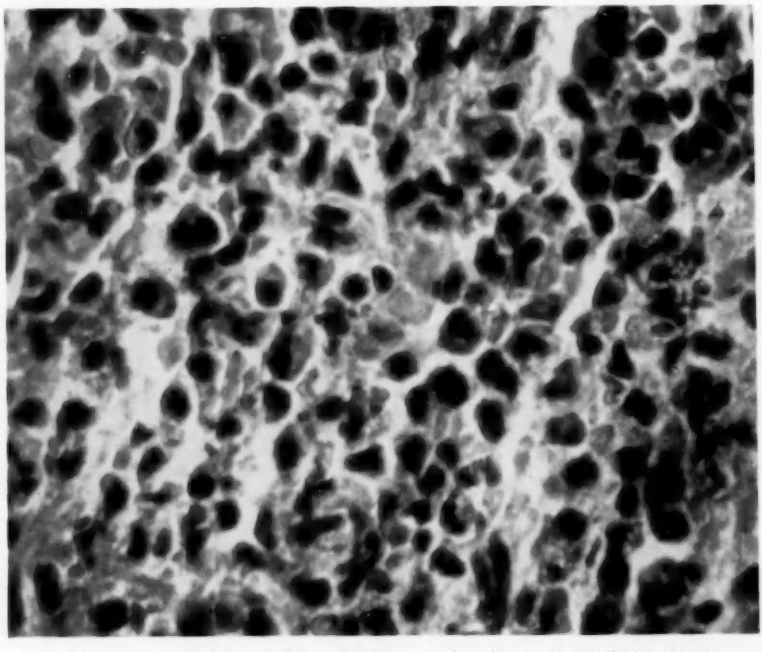

FIG. 4. Excessive numbers of plasma cells in section from a case of chronic congestive splenomegally associated with schistosomiasis.

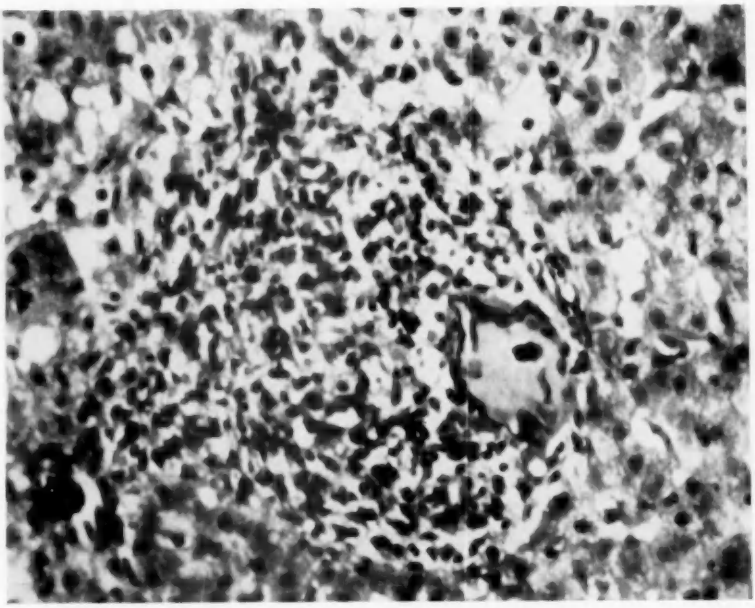

FIG. 5. Schistosome ovum surrounded by inflammatory cells in between the hepatic lobules. Section from liver biopsy in a case of chronic congestive splenomegaly.

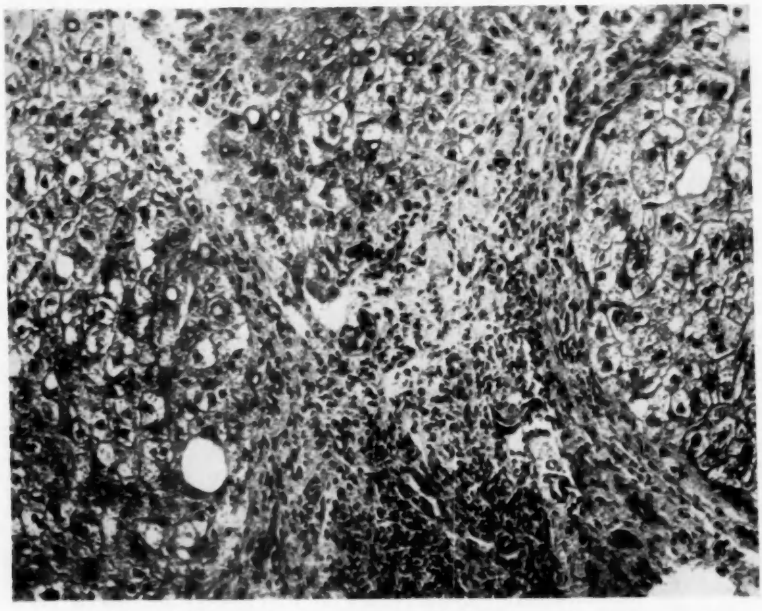

FIG. 6. Portal cirrhosis in a case showing chronic congestive splenomegaly.

FIG. 7. Infarction in a case of chronic lymphatic leukemia.

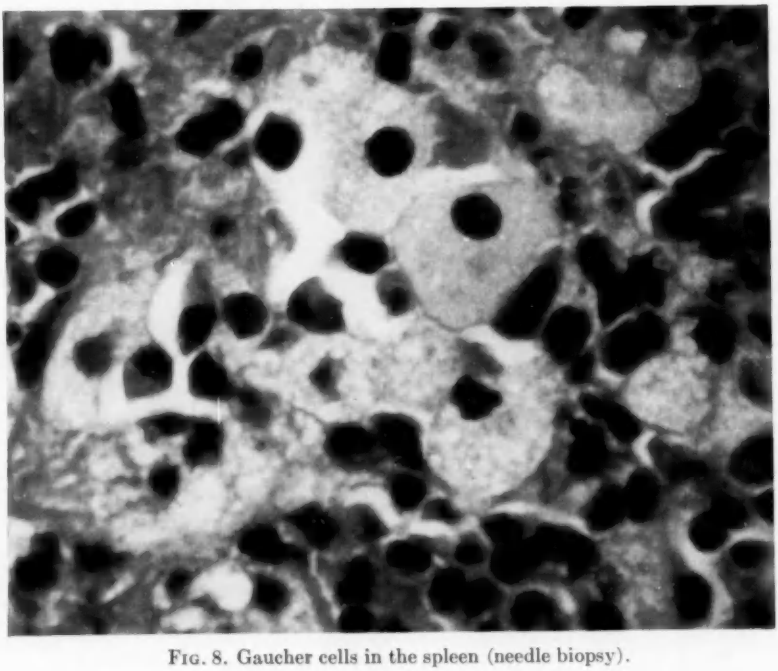

FIG. 8. Gaucher cells in the spleen (needle biopsy).

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PAPULAR SKIN LESIONS IN WHICH SCHISTOSOME EGGS WERE FOUND

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HISTORY OF CASE

Our patient was a girl 12 years old, complaining of multiple itching swelling over the trunk and upper part of the thighs, admitted to hospital on February 14th, 1955. There was a past history of urinary bilharziasis 7 years ago, treated by a course of antibilharzial injections and cured, but urinary troubles recurred after that.

One day in the morning, a year previous to admission, she felt a burning sensation in the skin of the front of the chest, abdomen, thighs and upper part of the neck. The color of the skin was not changed. In the afternoon of that day small reddish itchy papules appeared in the above areas spontaneously. After a few days the papules changed to vesicles, then pustules; then ulceration occurred, discharging yellowish green pus for about one month during which time she received penicillin injections until the ulcers healed, leaving firm keloid swellings at their sites.

Examination

The primary lesions were papules 1 x 0.5 cm., firm, not tender, pinkish white, keloid in nature, surrounded by erythema; the skin between was normal. There were also smaller conical papules having the same color as the skin, accompanied by abrasions from scratching. The number of lesions in the different sites were: front of the chest, 8; abdomen, 53; glutea, 9; front of right thigh, 20 and 3 behind; front of left thigh 28 and 10 behind. The scar of the biopsy healed by keloid formation (Fig. 1).

The patient was referred to the physician and his report was as follows:

Chest: free; heart: impaired note over the second left intercostal space and accentuated second pulmonary sound; abdomen: the spleen one finger below the costal margin, the liver two fingers below the costal margin.

Investigations

Urine: traces of albumen, pus cells, red blood corpuscles and living *Schistosoma haematobium* eggs were present.

Stools: negative for parasite cysts and eggs.

Blood picture: hb 89%; leucocytes 13,600; differential count: polymorphs 26%, lymphocytes 22%, monocytes 7%, eosinophils 44%, basophils 1%. Sedimentation rate before specific treatment: first hour 18, second hour 26. Sedimentation rate two days after starting specific treatment: first hour 20, second hour 42.

FIG. 1. Patient before treatment showing papular skin lesions of schistosomiasis

X ray report: lungs clear, no enlargement of the pulmonary conus, and no other bilharzial manifestations in the lungs.

Biopsy report: the specimen received consists of a piece of the skin and the underlying subcutaneous tissue. The dermis contains numerous foci of round cells, and in the center of one of these a section of a schistosome egg and a foreign body giant cell are seen (Fig. 2 and 3). (When other serial sections were made we found in them remnants of two other eggs.)

Treatment

The patient used antipruritic lotions and ointments and received calcium and vitamin C injections with no appreciable improvement. When antibilharzial

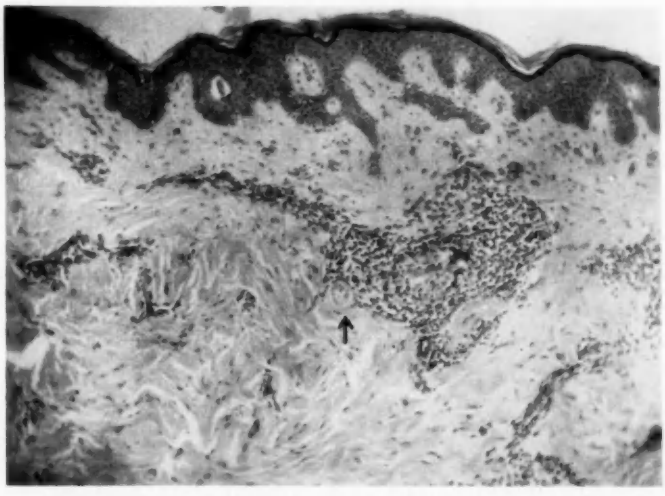

FIG. 2. Low-power section of a cutaneous papule showing egg of *S. haematobium*

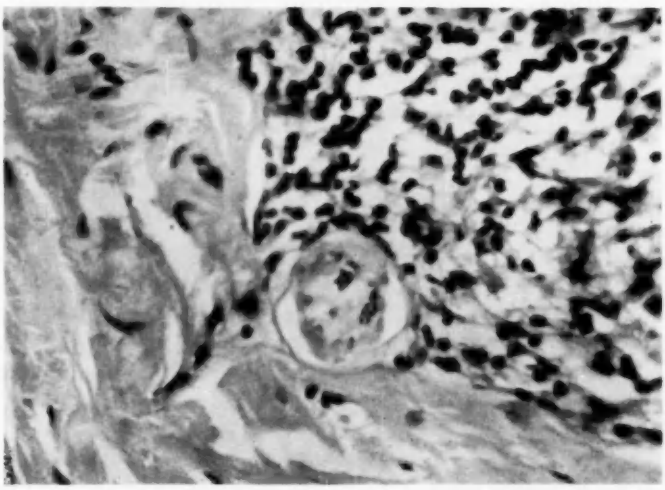

FIG. 3. High-dry magnification of the egg in Fig. 2

treatment was given, itching increased and eczematous reactions appeared around the old lesions for about a week, then gradually disappeared and the original papules became gradually smaller in size, leaving a slight pigmentation behind.

Reviewing the previous literature, we found that schistosomal affection of

the skin occurs in three stages. (1) The first is a cercarial dermatitis mainly due to nonhuman cercariae, especially those of birds. (2) Skin lesions of an anaphylactoid character appear in the allergic stage,, "katayama disease", 4–6 weeks after the original infection when the cercariae of human schistosomes reach maturity in the liver (early toxic stage); it follows infection with any of the human schistosomes but mainly with *japonicum* and *mansoni* rather than *haematobium*, and its features are: fever, urticaria, joint pains, bronchitis, enlarged liver and spleen, diarrhea, and high eosinophilia. The symptoms clear up usually after a few weeks, to be followed by the excretion of eggs in the stools and urine. (3) Dermatitis in the later stages of the disease is caused by embolic eggs which provoke inflammatory and fibrotic lesions in the skin appearing as papular or nodular eruptions which in severe cases lead to papillomatous, warty or acuminate condyloma-like growths. Gelfand (1950), in Rhodesia, described a bilharzial involvement of the vulva which manifested itself as an inflammatory swelling with nodules beneath the skin, progressing to a papillomatous formation.

All three species of *Schistosoma* have been found to cause skin lesions. Garcia, Navarro and Bautista (1941) reported a case of cutaneous schistosomal lesions containing *S. japonicum* eggs. Black (1946) reported 4 cases of cutaneous schistosomiasis, caused by *S. haematobium*, which appeared during treatment for vesical bilharziasis. Fishbon (1946) reported a case in which eggs of *japonicum* were demonstrated in multiple skin lesions. Barlow and Meleney (1949) reported schistosomal involvement of the skin in a voluntary infection with *S. haematobium*. El Zawahry (1955), in Cairo, has studied 10 cases of schistosomal granuloma in the last 10 years. They were papillomatous masses, largely in the perianal and genital regions, and were late lesions in which eggs were found.

Faust (1948) suggested the following theories to explain the ectopic lesions: (1) metacercariae develop into adults, laying eggs at or near the site of penetration, or (2) adults travel against the venous blood into collateral vessels.

COMMENT

Schistosome eggs are not found in every papule but accidentally in one or two; all papules diminish after antibilharzial treatment. After such treatment eczema-like reactions appeared around the old lesions in our patient, possibly due to toxins liberated by the action of the drug on the living worms and/or its eggs.

SUMMARY

A case of urinary bilharziasis in a 12-year-old girl developed a papulopustular eruption involving the skin of the chest, abdomen, thighs and gluteal region. Skin biopsy showed a schistosome egg in the center of a focus of round cells. Following treatment eczematous reactions appeared around the skin lesions, which gradually disappeared, leaving a slight pigmentation behind.

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THE PRODUCTION OF ANTIBODIES IN RABBITS INFECTED WITH IRRADIATED *TRICHINELLA SPIRALIS* LARVAE[1]

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Tyzzer and Honeij (1916) found that irradiation of trichinous muscle affects adversely the infectivity of *Trichinella* larvae. These results have been confirmed and extended by several investigators (Schwartz, 1921; Semrad, 1937; Levin and Evans, 1942; Evans, *et al.*, 1941; Alicata and Burr, 1949; Alicata, 1951). Recently, Gould and his co-workers (1953a, 1953b, 1954) recommended that pork intended for human consumption be irradiated as a means of controlling trichinosis. As shown by Evans and his co-workers (1941), irradiated larvae develop to adulthood in the small intestine but fail to produce second generation larvae which migrate to the musculature. Therefore, a person ingesting irradiated, infected pork will be spared the pathogenicity associated with the muscular phase of the infection. Magath and Thompson (1955) reject Gould's suggestion, arguing that, since irradiated larvae do not stimulate immunity against subsequent infections, man and hogs would become susceptible to severe clinical infections with a resulting increase in the mortality rate following ingestion of non-irradiated *Trichinella* larvae. This is in apparent contradiction to the results reported by Levin and Evans (1942) who observed formation of an acquired immunity following infections with irradiated larvae.

The most reliable procedures for the serological diagnosis of trichinosis in man are the flocculation and the complement-fixation reactions (Norman, *et al.*, 1956). The antigens for these reactions are extracted from *Trichinella* larvae after they have become encysted in the muscles. In view of the observations reported by Oliver-González (1941), it is generally believed that there is dual antibody production by the host; one type directed against the larvae of the parasite in the host muscle, the other against the adults in the small intestine. Therefore, it was deemed important for theoretical and diagnostic considerations to set up a study to determine whether or not anti-larval antibodies are produced in sufficient amounts following one or more inoculations with irradiated larvae to be detectable by the flocculation and/or the complement-fixation tests. Furthermore, attempts were made to determine whether the intestinal phase or the muscular phase of infection are sufficient to elicit a detectable serological response, independently of each other.

MATERIALS AND METHODS

Young adult rats were fed 3,000 washed *Trichinella* larvae each to supply fresh larvae for animal inoculations and for the preparation of antigens. Between 1 and 2 months after infection, the rats were killed and the larvae were separated from the muscles by artificial digestion in a pepsin-hydrochloric acid mixture.

[1] With the technical assistance of Misses Dorothy S. Allain and Nell M. King.

The *Trichinella* larvae were irradiated in distilled water in petri dishes, 50 mm. in diameter, with the top removed during irradiation. Irradiation factors were 250 KV, 15 MA, intensity of 200 r per minute. The dose rate was determined in air with a Victoreen condenser, "R" type, Model 70. The larvae were kept in distilled water just before, during, and just after irradiation. Two doses were used: 13,000 and 20,000 roentgens. Viability of larvae irradiated in vitro was tested by feeding approximately 5,000 larvae to each of a number of previously uninfected white rats. One-half of the rats were examined 7 days after inoculation for the presence of adult trichinae in the intestines. The others were sacrificed 30 days after inoculation. The diaphragm was examined microscopically and the entire carcass was artificially digested and examined for the presence of larvae.

The rabbits used in the immunizing experiments were adults varying in weight from 2,000 to 4,000 grams at the beginning of each experiment. Some of the rabbits were given, intragastrically, variable numbers of washed irradiated larvae. Control animals were given a similar quantity of non-irradiated larvae which had been handled in a similar way. In all cases, normal development and heavy infections resulted after intragastric inoculation with the non-irradiated larvae. Other groups of rabbits were given variable amounts of larvae by intravenous, subcutaneous and intramuscular routes. The syringe needle was dried before puncturing the rabbits and a few drops of collodion were placed at the site of needle introduction in order to prevent the animals from ingesting a few larvae which might have escaped at the site of the inoculation. Following each inoculation, the animal was immobilized until the collodion drops formed a completely dry crust.

Serum specimens were obtained from the rabbits by bleeding them just before inoculation and every week thereafter up to the end of the experiments. All of the sera were tested by the bentonite flocculation reaction. Some representative serum specimens were also tested by the complement-fixation reaction. The rabbits were bled, routinely, from the marginal ear vein. At the end of each experiment, all the animals were sacrificed. Samples of rabbit muscle were examined by press preparation methods. In addition, not less than 300 grams of muscles, including the diaphragm, the tongue and the masseters, were digested from each rabbit and examined for the presence of larvae.

An acid-soluble extract of trichina larvae was used for the bentonite flocculation test (Melcher, 1943). The preparation of the antigen and the performance of the test were the same as described previously (Sadun and Norman, 1955a, 1955b). A crude larval antigen was used for the complement-fixation test (Norman *et al.*, 1956).

In summarizing the results for tabular presentation, the geometric means of titers (GMT) were obtained. The geometric mean is the "appropriate average when the data are limited at one end of the range and unlimited at the other, and there tends to be a constant rate of change from one value to the next", i.e., form a geometric progression (Kenny, 1947). In such cases, "the geometric mean will give the correct answer, while the arithmetic mean will not. Its value is not quite so greatly influenced by extreme items as are the values of the quad-

ratic, arithmetic, and harmonic means" (Waugh, 1943). The GMT was obtained by the formula

$$\text{GMT} = \frac{c}{a}\,\text{antilog}\left[(\log c)\left(\frac{-\sum Si}{n}\right)\right]$$

in which c represents the dilution factor (2); 1/a represents the original dilution (5) and Σ Si/n represents the average progressive dilution scores. In applying this formula, scores 1, 2, 3, etc., were given for sera with titers of 1:5, 1:10, 1:20, etc. In estimating the geometrical means of the titers, those sera which were negative at a titer of 1:5 were arbitrarily given a titer of 1:2.5, that is, half way between 0 and 5.

RESULTS

1. Single inoculation by ingestion. Three experiments were undertaken to study whether or not antilarval antibodies are produced in detectable amounts following a single ingestion of irradiated *Trichinella* larvae (Table 1).

In the first experiment, 6 rabbits were each given 10,000 larvae by mouth which had been irradiated with 20,000 roentgens. Five controls received the same number of non-irradiated larvae. All the sera gave negative flocculation reactions before, and three weeks after, inoculation. Four of the control rabbits became positive four weeks, and one five weeks, after inoculation. The GMT increased regularly up to the end of the experiment, seven weeks after inoculation (1:40). All the sera from the rabbits receiving irradiated larvae remained negative throughout the experiment. No adults were found in the intestines of rats which received 5,000 irradiated larvae each, and no larvae were found after digestion of the rat carcasses 30 days after inoculation. Likewise, no larvae were recovered after the digestion of 3,800 grams of muscle from the six rabbits receiving irradiated larvae.

In the light of these results, another experiment was set up to study whether a much heavier inoculum with irradiated larvae might provide sufficient stimulation for the production of detectable amounts of antibodies. In the second ex-

TABLE 1

Serum antibodies in rabbits following a single ingestion of irradiated and non-irradiated Trichinella *larvae*

Experiment number	No. of rabbits	Larvae given per animal × 1,000	Radiation dose (roentgens) × 1,000	Geometric mean of the titer (reciprocal)							
				Time in weeks from ingestion of larvae							
				0	1	2	3	4	5	6	7
I	6	10	20	—	—	—	—	—	—	—	—
	5	10	—	—	—	—	—	5	20	23	40
II	6	100	20	—	—	—	—	—	—	—	—
	5	100	—	—	—	4	6	8	23	61	80
III	6	50	13	—	—	—	—	—	—	—	—
	7	50	—	—	—	4	13	30	131	160	145

periment, six rabbits were each given 100,000 larvae which had been irradiated with 20,000 roentgens. Each of 5 controls received the same number of non-irradiated larvae. All the sera gave negative flocculation reactions before, and 1 week after, ingestion of the larvae. Whereas the sera from the rabbits receiving irradiated larvae remained negative throughout the experiment, of those receiving non-irradiated larvae, two became positive two weeks, one three weeks, one four weeks, and one five weeks, after inoculation. They all remained positive throughout the experiment. The GMT increased somewhat regularly up to the time the animals were sacrificed (1:80). At necropsy, no worms were found in the rabbits nor in the control rats that had received irradiated larvae.

A third experiment was set up to test whether the lack of positive results obtained in the previous two experiments following ingestion of irradiated larvae might have been due to the fact that 20,000 roentgens had rendered the larvae non-infective. Obviously, the desired dosage for a study of this kind is one which will least affect the development of the *Trichinella* adults in the host's intestine and, at the same time, will prevent the formation of larvae destined to invade the muscles. In view of the fact that Gould and his coworkers (1954) found sterility of all the female adults when the larvae were subjected to a dose of 11,830 r, in the present experiment six rabbits were inoculated with larvae which had been exposed to 13,000 r. Five control rabbits received equal numbers of non-irradiated larvae. As in the previous experiments, all the sera from the animals receiving irradiated larvae gave negative flocculation results. On the other hand, four of the five controls gave positive reactions two weeks, and the fifth became positive four weeks, after inoculation. All remained positive up to the end of the experiment and their GMT increased gradually up to six weeks following ingestion of the larvae (1:160). Immature *Trichinella* adults were recovered 7 days after inoculation from two rats which had received 5,000 irradiated larvae. No eggs or young embryos could be recognized in the uteri of the females. No larvae were found after digestion of the rats and 2,700 grams of muscle from the six rabbits which had received irradiated larvae. Complement-fixation reactions of sera from rabbits fed irradiated larvae were negative, whereas from the controls receiving untreated larvae, complement-fixation reactions were positive at relatively high titers.

2. Multiple inoculations by ingestion. Two experiments were designed to find out whether demonstrable amounts of antilarval antibodies could be obtained in rabbits which had been repeatedly infected with irradiated larvae (Table 2).

In the first experiment, five rabbits were each given, at weekly intervals, 3 doses of larvae irradiated with 13,000 r. In this and the next experiment, no control rabbits were inoculated since it had already been shown in the previous series of experiments that even single doses of 10,000 non-irradiated larvae by ingestion are sufficient to elicit significant levels of reactivity. Three of the rabbits became positive two weeks, one three weeks, and one four weeks after the first ingestion of irradiated larvae (one, two, and three weeks after re-inoculation, respectively). The antibody titer remained somewhat low and reached the peak on the fifth week after the first inoculation (GMT 1:9). Seven weeks following

TABLE 2

Serum antibodies in rabbits following multiple ingestions of irradiated and non-irradiated Trichinella *larvae*

Experiment number	No. of rabbits	Larvae given per animal × 1,000	Radiation dose × 1,000 (roentgens)	Geometric mean of the titer (reciprocal)												
				Time in weeks from first ingestion of larvae												
				0	1	2	3	4	5	6	7	8	9	10	11	12
I	5	60; 30; 50 (1 wk. apart)	13	—	—	4	5	5	9	4	—	—	—	—	—	—
II	6	100; 100	20	—	—	—	—	—	—	—	—	7	5	4	3	—
	6	10; 10 (6 weeks apart)	20	—	—	—	—	—	—	—	—	3	3	—	—	—

the first inoculation (five weeks after the last inoculation), all the rabbits had reverted to negative reactions. Control rats were fed irradiated larvae following each of the three rabbit inoculations. At necropsy, 7 days after ingestion, immature *Trichinella* adults were recovered from the intestines of these rats. As in the previous experiments, no eggs or young embryos could be recognized in the uteri of the immature females. A few larvae were recovered from the muscles of one of the rats sacrificed 30 days after inoculation. Digestion of 3,200 grams of muscles from five rabbits was performed at the end of the experiment. No larvae were found in three rabbits, 278 larvae were obtained from one rabbit (500 grams of muscle digested) and 91 larvae were obtained from another rabbit (900 grams of muscle digested).

Although positive titers were obtained from three rabbits in which no larvae were recovered, the presence of larvae in one rat and two rabbits might raise the question whether or not the serum reactivity observed in these experiments was due exclusively to the presence of the adult worms in the intestine or to some larvae which might have invaded the muscles. Therefore, another experiment was set up in which larvae were subjected to 20,000 r before being fed to rabbits. Six rabbits received two heavy doses (100,000 each) and six rabbits received two relatively light doses (10,000 larvae each) with an interval of 6 weeks between inoculations by feeding. All the sera were negative up to one week following reinoculation. In the group receiving heavy inocula, five rabbits became positive in the second week following the second inoculation and the other rabbit became positive in the third week. Most of these animals remained positive for periods varying between 1 and 4 weeks. By the twelfth week (6 weeks following the second inoculation) all had reverted to negative. Their antibody titer was relatively low, reaching the peak during the first week of positivity (GMT 1:7) and decreasing up to the end of the experiments. In the group receiving light inocula, only 2 rabbits became positive two weeks following reinoculation. One of them remained positive for 2 weeks whereas the other reverted to negative the following week. After the tenth week, all were negative up to the end of the experi-

TABLE 3

Serum antibodies in rabbits following multiple extra-intestinal inoculations of non-irradiated Trichinella *larvae*

Group No.	No. of rabbits	No. of inoc.	Larvae given per animal × 1,000 (3 wks apart)	Inoc. route	Geometric mean of the titer(reciprocal)														
					Time in weeks from first inoculation of larvae														
					0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	8	2	135 to 200	I.V.	—	—	3	5	6	8	14	17	25	20	18	18	14	28	20	20
II	4	2	335 to 575	Sub-cut.	—	—	—	6	9	23	7	11	40	35	20	20	40	28	80	57
III	2	3	475 to 575	I.M.	—	—	14	20	14	14	40	28	40	28	57	160	80	80	40	40

ment. No worms were recovered from the rats which had received 5,000 larvae irradiated with 20,000 r. Digestion of 6,300 grams of muscle from the 12 rabbits in this experiment failed to reveal any nematode larvae. Complement-fixation reactions of sera from rabbits inoculated with irradiated larvae were negative after the first inoculation and were positive but with low titers shortly following re-inoculation.

3. Extra-intestinal inoculations. In the previous series, comparisons were made of the antibody levels of reactivity in the animals stimulated by the intestinal phase alone. In the following series, rabbits were given *Trichinella* larvae intravenously, subcutaneously, and intramuscularly so that only the larval muscular phase would be present. A total of 14 animals were included in this series (Table 3). Eight of them were given between 135,000 and 200,000 larvae intravenously in two inoculations three weeks apart. One rabbit became positive two weeks after the first inoculation, three after three weeks, and one after four weeks. Five weeks after the initial inoculation (two weeks after the re-inoculation) all were positive. The GMT increased somewhat regularly up to the eighth week (1:25) and then it remained more or less constant. At the end of the experiment, the animals were necropsied and samples of muscles were digested. The muscles of the ears were digested separately. Larvae were recovered, after digestion, from six of the eight rabbits in muscles other than the ear muscles. The larvae varied in number and in appearance. Many larvae were alive and appeared to be encysted; several were dead and showed signs of internal deterioration, the cuticle being the only portion left. In one rabbit, 27 adults, as well as many larvae, were recovered from muscles other than the ear muscles. The adults were well developed and sexually differentiated (13 males and 14 females). Embryos were seen in the uteri of some of the females. The males varied in size between 1.0 and 1.5 mm. with a mean of 1.2 mm. The females varied from 2.5 to 3.2 mm. with a mean of 2.8 mm. The curve of antibody titer of this rabbit was essentially similar to that of the others in the group.

Four rabbits were given between 335,000 and 575,000 larvae subcutaneously in three doses with a three week interval between inoculations. Two animals became positive three weeks, one four and one five weeks after the first inoculation. The GMT increased up to the eighth week (1:40) after which time it remained more or less constant. At necropsy, larvae were recovered, after digestion, from

the subcutaneous tissues of all the rabbits. Most of the larvae were dead and degenerated. A few living larvae, however, were also recovered from two animals. In one, they varied in size from 0.66 mm. to 1.5 mm. with an average of 1.14 mm. In the other, they varied from 0.66 mm. to 1.33 mm. with an average of 1.00 mm.

Two rabbits were given between 475,000 and 575,000 larvae intramuscularly in three doses with a three week interval between inoculations. Both animals became positive after three weeks and remained so up to the end of the experiment. The GMT increased up to eleven weeks after the first inoculation (eight and five weeks after the second and third inoculation, respectively) after which it began to decrease. No parasites, either living or dead, were recovered after muscle digestion at necropsy. Representative sera from each group gave positive reactions when tested by the complement-fixation test.

DISCUSSION

In the present investigation, rabbits which received single inoculations by ingestion of irradiated *Trichinella* larvae, even when very large doses were employed, failed to produce anti-larval antibodies in sufficient amounts to be detectable by the flocculation and complement-fixation tests. On the other hand, the intestinal phase of infection alone (produced by sterile adults) stimulated low antilarval antibody titers for short periods of time, provided more than one exposure took place. Conversely, as was to be anticipated from theoretical considerations, the muscular phase alone (produced by extra-intestinal larval inoculations) stimulated the production of antilarval antibodies in relatively high titers. Consistently higher levels of reactivity were obtained in rabbits with both the intestinal and muscular phases of infection (produced by normal non-irradiated larvae given intragastrically).

Some information related to the physico-chemical requirements of *T. spiralis* was obtained from the results of the extra-intestinal inoculations. At necropsy, six of the rabbits inoculated intravenously were found to harbor living larvae within the muscles. Some of these larvae appeared to be encysted. In one animal, several adults had developed and had become sexually differentiated in an environment very different from the normal habitat of *Trichinella* adults. On the other hand, no living larvae were recovered from the rabbits which had been inoculated intramuscularly. An interpretation of these findings is not possible at the present time.

Gould and his co-workers (1953a) recommended the use of commercially irradiated pork as a means of protecting humans from infection. If such a recommendation should be widely adopted, a re-evaluation of the significance of positive serology in trichinosis might become necessary. Consideration should be given to the present results which indicate that for a relatively short time positive flocculation and complement-fixation reactions with low titers may be observed in those individuals who repeatedly consume infected and irradiated pork that has been improperly cooked.

Attention is called to the fact that in the course of the experiments reported here, a few apparently normal larvae were recovered from the muscles of one rat

and two rabbits fed trichinae which had been exposed to 13,000 roentgens. None of the larvae exposed to 20,000 r were able to produce offspring. Whether or not larvae exposed to 20,000 r readily develop into adults and burrow into the mucosa could not be ascertained. These results are in agreement with other observations on the effect of radiation in producing artificial sterility in plants and animals and indicate that, although doses around 10,000 r are very effective in preventing reproduction of *Trichinella spiralis* (Evans *et al.*, 1941; Alicata, 1951; Hendricks, 1952; Gould *et al.*, 1953), a few organisms may fail to become sterile.

Of interest is the fact that rabbits stimulated with irradiated larvae showed an antibody titer to larval antigen despite the fact that they received little or no larval stimulation. These results are in agreement with and support those reported by Hendricks in mice (1952). This phenomenon could be due to the fact that a small antigenic stimulation by the infecting larvae was present before the adult stage was reached in the intestine, or the fact that the apparent qualitative difference between the different groups of antigens "may be solely a question of the relative proportions of the common antigens between larvae and adults" (Oliver-González, 1941; Chute, 1956).

It is suggested that a multitude of antigens are contained in each stage of the worms and that only a few are probably involved in studies such as the present one. The main antigenic differences between the larvae and the adults will probably reside in their respective balance of activity among various enzymes responsible for their different metabolic requirements in the muscles and the digestive tract. Studies are now in progress involving the isolation of antigens from the excretory and secretory products of the worms to provide a better understanding of the multiple antibody formation in *Trichinella spiralis* infection.

SUMMARY

Rabbits that received single inoculations by mouth of irradiated larvae of *Trichinella spiralis* failed to produce antibodies in sufficient amounts to be detected by the flocculation and complement-fixation tests. Multiple feedings with irradiated larvae produced a positive serology at low titers and of brief duration. Extra-intestinal larval inoculations stimulated the production of antibodies in relatively high titers. A re-evaluation of the significance of positive serology in trichinosis might become necessary if the recommendation of commercially irradiating pork as a means of protecting human beings from trichinosis should be widely adopted.

ACKNOWLEDGEMENT

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A FLEA AND RODENT CONTROL PROGRAM FOR PLAGUE PREVENTION IN THAILAND[1]

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The report of the Department of Public Health of Thailand for 1922 states that plague first appeared in Bangkok in 1904 and was believed to have been brought by Indian traders from Bombay. Bangxang (1948) has since expressed the opinion that plague was imported by the sea route from China but the consensus among officials of the Thailand Ministry of Public Health is that the more probable origin was Bombay.

According to the 1922 report, a total of 1,722 plague cases occurred in Bangkok from 1904 to 1920. Although no records of plague in the provinces are available before 1913, the disease was first found in Rat Buri about two years after its appearance in Bangkok. After 1913 plague occurred in Khorat (Nakhon Ratchasima) nearly every year. In 1917 there was a severe epidemic in that city, with more than 500 deaths. Buildings in large areas of the city were torn down and rebuilt under the direct supervision of the Department of Health and no serious epidemic has occurred since. During the five years following the Khorat epidemic, the worst sections of Nakhon Sawan, Phitsanulok, Lampang, and Hua Hin were similarly reconstructed as a preventive measure.

The Public Health Vital Statistical Record of Thailand for 1953 shows that 4,760 cases of plague and 3,439 deaths were reported from 1913 to 1952 (Fig. 1). The cases were invariably of the bubonic type (Bangxang, 1948). Plague was reported yearly except for the period from 1935 to 1937 when it was apparently absent. According to Park (1941), plague reappeared near the end of 1938 in the northwestern province of Tak, and spread eastward and then south to Bangkok which became reinfected in 1940. Only a few cases were reported from Bangkok at that time and none have since been reported. On the other hand, plague became entrenched in the provinces, but, according to Dr. Pramern Chandavimol, Chief of the Division of Communicable Disease Control, it occurs in a milder form than that reported before 1938.

Until the end of 1951, plague control measures had been instituted only after human cases were detected. At that time, however, the Department of Health decided to make an effort to prevent human cases. Permanent laboratories for the detection and control of the rat-borne disease were established in all the endemic plague areas of Thailand and sanitarians from the Division of Communicable Disease Control were assigned to them. (Sanitarians are officials who have been trained by the Ministry of Public Health to supervise the operation of second class health centers of which there is usually one in each rural district

[1] Parts of this paper appeared in the Bulletin of the Thai Department of Public Health in February, 1954.

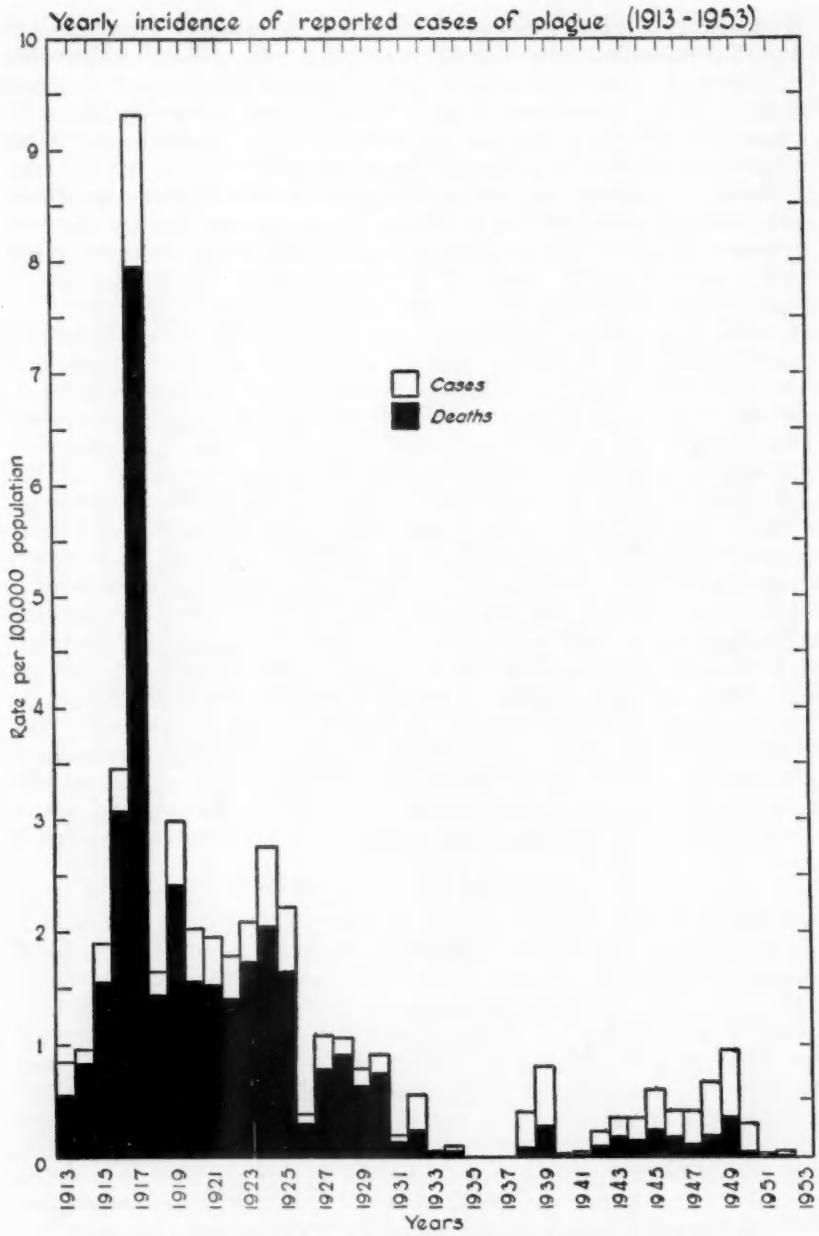

FIG. 1. Yearly incidence of reported cases of plague (1913–1953)

of Thailand). The sanitarians were first trained at the Banpong laboratory by the writers who then assisted them on their projects for various periods at Nakhon Sawan and Khorat. The purpose of the laboratories was not experimental but to apply established procedures of flea and rodent control to prevent the occurrence of plague, and for that reason the Division of Communicable Disease Control did not want an untreated comparison area.

During 1952, laboratories were established in the cities of Banpong, Khorat, and Nakhon Sawan, which were selected because of their location in known endemic plague areas (Fig. 2). Banpong was in the center of an endemic area in

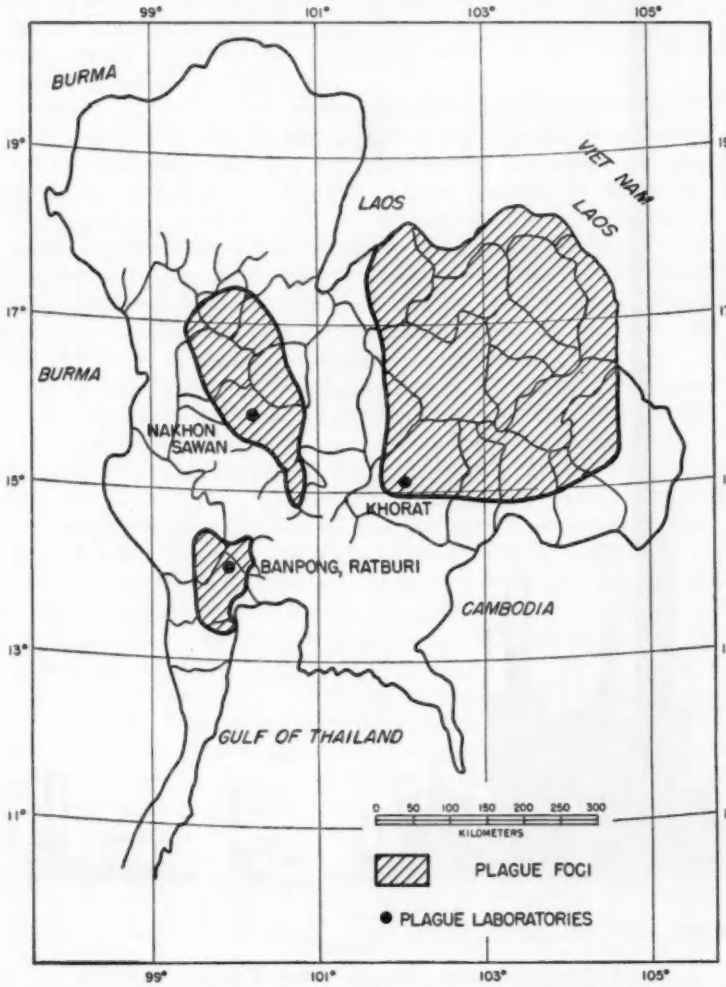

FIG. 2. Map of Thailand showing the location of plague foci and plague laboratories. Provincial lines are shown only for those provinces within the plague foci.

West Central Thailand which included four provinces; the Khorat endemic area included the 15 Northeast provinces; while the Nakhon Sawan laboratory served five provinces in North Central Thailand. Central Thailand is a great plain on which the western rim, formed by the Tenasserim mountain range, is overgrown with deciduous forest. Most of the rest of the region is intersected with an endless succession of rice fields. Northeastern Thailand slopes from the high Phetchabun mountains on the west to the southeast and is dominated by rice fields, although about one-third of its area consists of dispersed hilly areas which support deciduous forests.

Thailand is subject to the typical monsoon climate of Southeast Asia. There are three poorly distinguished seasons in the region of Bangkok which become progressively more distinct further north. The monthly tables of climatological data obtained from the Royal Thai Navy for 1951–1953 show little difference in mean temperatures between one of the selected areas and another, although Khorat was consistently cooler by one or two degrees than the other two. The cool season (21°–28° C.) lasts from October to February; the hot season (28°–32° C.) from March to May, and the rainy season with its intermediate temperatures from May to September. The humidities rise during the rains from the 60's and 70's in the hot quarter of the year, into the 70's and 80's. Nakhon Sawan was 5–15% drier at all seasons than the other two. These seasonal differences in temperature and humidity undoubtedly influence the flea densities and the occurrence of plague. Park (1941) stated that cases of plague usually occurred between December and March, and Bangxang (1948) definitely defined the plague season as September to March. Sharif (1951) pointed out the correlation between flea prevalence and human plague in Bombay, India which has the same monsoon season as Thailand; flea populations and human cases increased from December to February, with a sharp decrease in both during the summer months, April to June. Similarly, plague tends to occur most commonly in the dry season (Fig. 3), and in deforested areas in Thailand, in contrast to malaria which occurs most frequently in forest areas during the rains.

MATERIALS AND METHODS

In general the following procedures were established at each of the three laboratories. Domestic rats were live-trapped daily from as many cities and villages as possible within the endemic area. Methods for obtaining and examining rodents and their ectoparasites were similar to those explained in "Rat-Borne Disease Prevention and Control" (U. S. P. H. S., 1949) with the exceptions that follow. Because multiple-capture wire cage traps were used, domestic rats from the same locality were placed in the same flea-proof bag; thus the percentage of rats infested with fleas could not be computed. In the fields or forests of the endemic area, animals other than domestic rats were killed with shotguns or were live-trapped with single-capture wire cage traps and these animals were placed separately in the flea-proof bags.

Ectoparasites were removed by beating the hosts with a three-cornered file over an enameled pan as practiced at the San Francisco Field Station of the United States Public Health Service (See Figure 4 in Eskey and Haas, 1940).

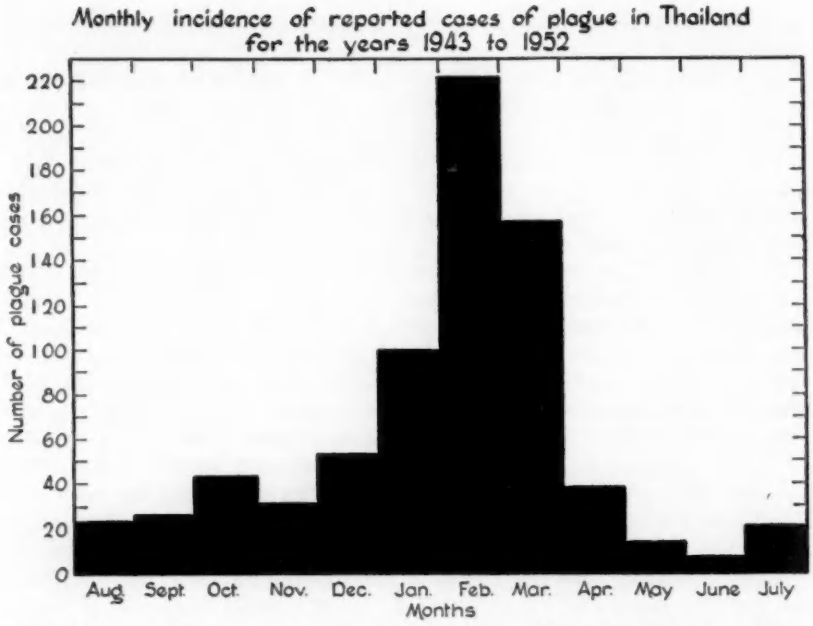

FIG. 3. Monthly incidence of reported cases of plague in Thailand for the years 1943 to 1952.

The lots of domestic rodents and their fleas were counted and identified while the forest or field-caught hosts were catalogued individually. The domestic rat fleas were pooled and macerated in 2 per cent saline for inoculation into culture media (Eskey and Haas, 1940). The mites and lice from the domestic rats and all the ectoparasites from the other hosts were preserved in 75 per cent alcohol for identification. Skins were prepared from the hosts for identification.

The spleens of all domestic rodents and some other hosts were excised and placed in a clean covered container until macerated for inoculation. Routine bacteriological procedures were used for isolating plague bacilli (Pollitzer, 1954). Occasionally etherized antigens were prepared in the manner described by Larson *et al.* (1951) from tissues of live-trapped rodents and sent to Dr. Larson of the P. H. S. Rocky Mountain Laboratory at Hamilton, Montana, for precipitin tests.

The plan usually followed was that houses in cities or villages were treated with DDT when human cases of plague occurred or when plague was found bacteriologically in the domestic rat *R. exulans* or the flea *X. cheopis*. The DDT treatment usually consisted of dusting rat-runs and harborages with 10 per cent DDT in calcium carbonate (U. S. P. H. S., 1949). Hand dusters or shakers were employed to distribute the DDT powder. Villages, cities, or sections of cities, were also treated or re-treated with DDT when surveys indicated that the

particular *X. cheopis* index was above 1.0 for any certain month. The 1.0 limit was selected in view of Hirst's (1926) postulate that where *X. cheopis* was the only important vector, there was no danger of an epidemic as long as the *X. cheopis* index remained below 1.0. An attempt was made to obtain by live-trapping at least 100 *R. exulans* from each village or city surveyed and to survey the major cities and villages in each district within the endemic area as often as possible.

The DDT treatment was followed by a rat poisoning program. Poisons employed were 1080 (sodium fluoroacetate) and Warfarin; 1080 was mixed with boiled rice and Warfarin was mixed with dry rice bran and peanut oil.

OPERATIONS

Plague control measures. Two epidemics of plague immediately preceded the establishment of the regional laboratories. The places where these occurred were the only ones where five or more cases of a plague-like disease appeared at one time during the course of these studies.

In northeastern Thailand early in November, 1951, an epidemic which was probably plague occurred in Ban Lat and two adjacent villages in the Phukhieo district of Chaiyaphum province. Of a total of 28 cases, seven with buboes died before treatment could be given. The remaining 21 cases recovered following treatment with streptomycin and sulfadiazine administered by the district health officer. A plague-control team from the Division of Communicable Disease Control found many dead rats and the spleens of two of these were inoculated into two guinea pigs which were sent to the Department of Health Laboratory in Bangkok. The guinea pigs died but *Pasteurella pestis* was not found in their tissues. Ban Lat and all villages within a radius of six kilometers were treated with DDT and domestic rats were poisoned. Treatment and poisoning continued through January, 1952, to include all villages in the district within a radius of 16 kilometers of Ban Lat. Domestic rats were live-trapped during January throughout the area, and the *Xenopsylla cheopis* index for Ban Lat was 0.7 for 105 *Rattus exulans* while 16 kilometers from Ban Lat in Ban Non Khun the index was 3.1 for 100 *R. exulans*. Possibly the latter index had not yet been affected appreciably by the DDT treatment which was under way in Ban Non Khun. In this connection, the Technical Development Division of the United States Public Health Service has shown that about five days after dusting, 99.3 per cent control of *X. cheopis* could be obtained (U. S. P. H. S., 1949). A brief survey of the same area was made in December, 1952, by the Khorat laboratory, but none of the villages were re-treated with DDT at that time. The *X. cheopis* index for Ban Lat was 0.6 for 56 *R. exulans* and that for Ban Non Khun was 1.1 for 35 *R. exulans*. The district sanitarian was instructed to continue trapping from the villages and to dust with DDT if the indices rose. No indication of plague was found bacteriologically from the rodents or fleas captured in Phukhieo district in either period.

Early in February, 1952, five cases of plague occurred in the Chinese section of Photharam, Rat Buri, a city in the endemic area of the Banpong laboratory.

The first case was a 43-year-old man who was sick five days, died, and was cremated before the case was reported to the health authorities. No doctor had examined him but according to the headman for that section of the city the patient had had fever and an enlarged right axillary lymph gland. The four other cases recovered after treatment with streptomycin and sulfadiazine, administered by the city health officer. Examination of the first patient's vacated bamboo house by the Banpong plague laboratory sanitarians on February 14 disclosed two mummified rats. The city of Photharam was dusted with DDT during February and domestic rats were live-trapped at the same time, giving an *X. cheopis* index of 1.1 for 112 *R. exulans*. This city was not surveyed again until August, 1952, at which time it was not re-dusted with DDT since the index was only 0.5 for 190 *R. exulans*. Plague was not isolated bacteriologically from the rats or fleas obtained from Photharam in either period.

During 1952 three more cases of a plague-like disease occurred in the Banpong area in the rainy season. The first two cases, from the same family, occurred in June in Luk Kae city, Kanchanaburi province, in a neighborhood where the *X. cheopis* index was only 0.5 from 35 *R. exulans*. Both cases recovered without treatment. Rats in the neighborhood were poisoned with 1080. The third case occurred in July in a village in Rat Buri province and district where the headman reported the case after the seven year old patient had died. No doctor had examined him but neighbors reported that several rats had died in the boy's house prior to his illness. The Banpong Unit treated the village with DDT and followed up with Warfarin poisoning.

RODENT AND FLEA CONTROL PROGRAM

The fleas found on the rodents caught in the houses were *Xenopsylla cheopis* and *Ctenocephalides felis* but the latter was found rarely, numbering less than one per cent of the total number of fleas obtained. Two species of lice, *Polyplax spinulosa* and *Hoplopleura oenomydis*, and the mites, *Laelaps nuttalli*, *L. sanguisugus*, *L. echidninus*, *Longolaelaps longulus*, and *Dermanyssus* sp., were found on the house rodents.[2]

The majority of the house rodents were *Rattus exulans concolor*; the mouse, *Mus* sp., numbered less than 5 per cent of the total collection from the Banpong area and approximately 20 per cent from the Khorat area. The field rats, *Rattus rattus* subsp. (near *jalorensis*, see Harrison and Traub, 1950), *Bandicota indica* subsp. and *Bandicota* sp., were also caught in houses, but numbered less than one per cent of the total collections of house rodents in all areas. In the Banpong area the house shrew, *Suncus murinus*, numbered less than one per cent of the total house collections, but was obtained from snap traps much more often than from cage traps.

From cities and villages within each of the endemic areas, the totals of live-trapped *Rattus exulans* were: 17, 998 for the Banpong area between February,

[2] Verifications on ectoparasite determinations were kindly made by Col. Robert Traub, MSC, (fleas); Major Hugh L. Keegan, MSC, (mites); and Dr. Phyllis T. Johnson, United States National Museum (lice).

1952, and September, 1953; 14,309 for the Khorat area between August, 1952, and September, 1953; and 16,246 for the Nakhon Sawan area between September, 1952, and September, 1953. These were all brushed and identified, their excised spleens and the *Xenopsylla cheopis* examined bacteriologically or serologically for the presence of plague and *X. cheopis* indices computed per village or city each month for dusting or re-dusting purposes.

To compare the results of control operations, the cities with regional laboratories, Banpong, Khorat, and Nakhon Sawan, were examined critically, since monthly data were available over a period of one year. Table 1 shows the number of live-trapped *R. exulans*, the number of *X. cheopis*, and the *X. cheopis* indices from each city for the period between August, 1952, and September, 1953. As seen from Table 1, the total average *X. cheopis* index for Banpong was above 1.0 while these indices for Khorat and Nakhon Sawan were below 1.0.

TABLE 1

Monthly totals of live-trapped Rattus exulans concolor, Xenopsylla cheopis *and the* X. cheopis *indices for Banpong, Khorat, and Nakhon Sawan*

Seasons	Year and month	Banpong[1]			Khorat[2]			Nakhon Sawan[3]		
		exulans	*cheopis*	Index	*exulans*	*cheopis*	Index	*exulans*	*cheopis*	Index
	1952									
Rains	Aug.	894	948	1.06	1106	695	0.63	—	—	—
	Sept.	428	478	1.12	834	497	0.60	1036	1161	1.12
	Oct.	482	361	0.75	445	428	0.96	1101	1282	1.16
	Nov.	284	232	0.82	313	165	0.53	623	612	0.98
Cold	Dec.	432	462	1.07	387	255	0.66	100	99	0.99
Plague	Totals	2520	2481	0.99	3085	2040	0.66	2860	3154	1.10
	1953									
	Jan.	528	700	1.33	473	282	0.60	264	248	0.94
	Feb.	326	661	2.03	488	373	0.76	392	292	0.75
Hot	March	389	735	1.89	381	266	0.70	515	300	0.58
	April	210	231	1.10	429	191	0.45	350*	203*	0.58*
	May	433	529	1.22	437	183	0.42	488	153	0.31
	June	605	721	1.19	259	199	0.77	700	250	0.36
	July	630	945	1.50	427	271	0.64	674	285	0.42
Rains	Aug.	845	880	1.04	1371	1980	1.44	895	360	0.40
	Sept.	667	441	0.66	681	925	1.36	615	206	0.34
	Totals	4633	5843	1.28	4946	4670	0.94	4893	2297	0.47
	Grand total	7153	8324	1.16	8031	6710	0.84	7753	5451	0.70

[1] DDT dusted Jan., 1952 (all houses); June, 1953 (15 houses); July, 1953 (50 houses).

[2] DDT dusted Dec., 1952 (20 houses); Sept., 1953, (5 houses); Nov., 1953 (all houses).

[3] DDT dusted Oct.–Dec., 1952 (all houses); Feb., 1953 (225 houses); Sept., 1953 (all houses).

* Plague was detected serologically from *R. exulans* spleens and *X. cheopis* in Nakhon Sawan on 6 April 1953.

BANPONG

Banpong, a city of 9,468 people and 1,835 houses, was completely dusted with DDT during January, 1952. The flea index began to rise above 1.0 in December but unfortunately the city was not dusted again until June, 1953, so that for most of the period under consideration it was essentially an untreated area with indices constantly above 1.0. During the rains of 1953 two sections of the city with many rats and high flea indices were re-dusted with DDT, 15 houses in June and 50 houses in July.

Between February and April, 1952, following the January treatment, 589 *R. exulans* and 599 *X. cheopis* were obtained by live-trapping from the city. Monthly *X. cheopis* indices showed a marked decline from a high index of 1.80 in February to the rather low index of 0.49 in April. The city was not live-trapped between April and August, 1952, so no indices are available for this period. When trapping was resumed, the index was found to be slightly above 1.0 during August and September and it then declined to a low of 0.75 in October. Then began a steady rise which reached a peak of 2.03 in February, 1953. Following the June and July, 1953, treatment a decline in the *X. cheopis* index was again observed, particularly in September, with an index of 0.66 (Fig. 4).

It is possible that the pronounced decline in the flea index in the spring of 1952 was in part the result of the January DDT dusting, particularly since the entire city was treated. However, although fleas reached a low point in Banpong in April, 1952, three months after DDT dusting, they also fell off sharply in April, 1953, without any further attempt at control (Fig. 4), and since April is the peak of the hot, dry season, it is probable that at least part of the decline in April, 1952, as well as the decline in April, 1953, reflected a seasonal fall in flea population in Banpong like that in Bombay (Sharif, 1951). The build-up in flea index between October and February, with the subsequent moderate decline, appears to be the pattern which can normally be expected under untreated conditions, with the index remaining relatively high until after the June and July dustings. The relatively low indices during the rains corresponded to those of the previous year and hence we cannot assume that the low September index was due to the summer dustings alone, since only 65 houses of the total 1,835 in the city were treated; it seems more probable that much of the reduction in flea index at this time was the result of the normal seasonal fluctuation in the flea population.

KHORAT

Khorat, a city of 42,562 people and 3,395 houses, was partially treated twice with DDT during the period of the study, in sections where the *X. cheopis* index rose above 1.0: a section of 20 houses in December, 1952, and the prison section of five houses in September, 1953. These were spots of particularly high flea indices. The last previous use of DDT in the city was in February, 1950, when the Division of Communicable Disease Control employed 50 men for ten days to treat the whole area with either DDT emulsion, a suspension of water-wettable DDT, or dust, each in a concentration of five per cent.

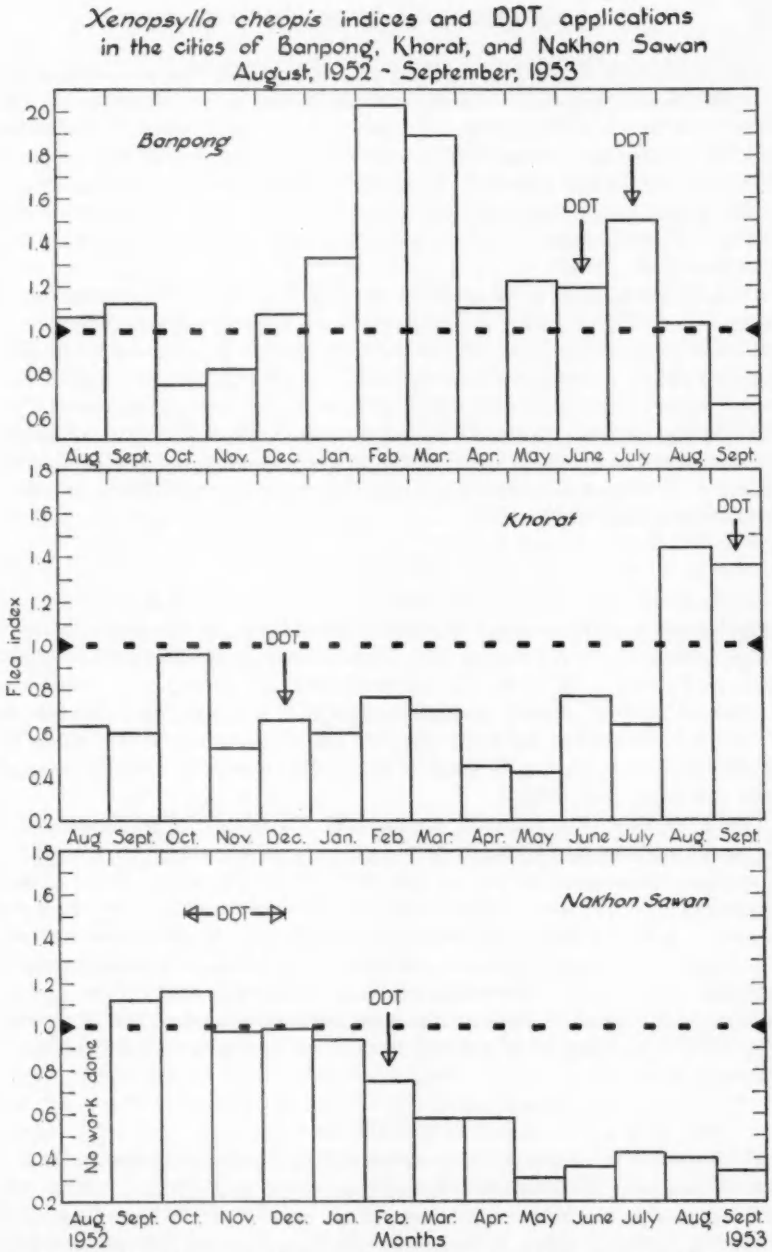

FIG. 4. Monthly *Xenopsylla cheopis* indices and DDT applications in the cities of Banpong, Khorat, and Nakhon Sawan for the period August, 1952 to September, 1953. A horizontal arrow indicates complete DDT application between the months shown and a vertical arrow indicates partial DDT application during that particular month.

Several factors may have combined to account for the relatively low *X. cheopis* indices found in Khorat during most of this period (Fig. 4). The treatment of 20 houses in December, 1952, could hardly have been sufficient in itself to keep the flea population under control in a city of over 3,000 houses. The climate of the Khorat area may be somewhat unfavorable for a large build-up of flea population, particularly the temperature, which was consistently lower during this period than that of the other two areas studied, though rarely more than 2 or 3° C.

While it seems unlikely that effects of the DDT treatment one and one-half years prior to the beginning of this study could have persisted to cause the low indices in Khorat, this possibility must be considered. Morlan and Hines (1951) reported effective control of *X. cheopis* and murine typhus three years after rat runs and harborages had been treated with the last of five applications of DDT. Considering, however, that treatment of the entire city of Banpong did not keep the flea index below 1.0 six months later, it seems more probable that *X. cheopis* indices in Khorat during this period represent natural oscillations in the flea population of that city.

NAKHON SAWAN

Of the three cities under study, Nakhon Sawan, a city of 30,496 people and 4,824 houses, was the only one completely dusted with DDT during the period under consideration. All houses were treated between October and December, 1952; in February, 1953, the market section of 225 houses which showed an *X. cheopis* index of above 1.0, was redusted, and although the index was well below 1.0 in September, 1953, the city was again completely treated with DDT to prevent a possible rise. Results of the latter treatment were not available when this paper was written.

The *X. cheopis* index began to decline after the first DDT application in the fall, and continued to drop steadily in succeeding months (Fig. 4). It is difficult to explain the lowered indices as the effect of climate alone. While Nakhon Sawan has generally a lower mean relative humidity than the other areas studied, and a mean monthly temperature intermediate between them, variations from 1952 to 1953 within the area of Nakhon Sawan itself did not appear to be sufficient to account for the marked decrease in monthly flea indices. It seems quite possible, therefore, that in this case DDT treatment of the city was responsible for reducing the *X. cheopis* population to the low level indicated by the 1953 indices.

DISCUSSION

Although human plague did not appear during the plague season in the areas studied, danger of transmission during that season was always present, especially when the flea index rose above 2.0 in Banpong in February, 1953. By comparing Figures 3 and 4, it can be seen that for Banpong between December, 1952, and April, 1953, the curve of the *X. cheopis* index above 1.0 closely parallels the curve for plague incidence for Thailand for the years 1943 to 1952.

Unfortunately, from the viewpoint of this study, the lack of human plague cases in all three areas made it impossible to demonstrate conclusively the effectiveness of DDT treatment for the prevention of plague. The contrast in flea indices between the essentially untreated area of Banpong and the treated area of Nakhon Sawan was striking but a correlation between flea indices and human plague could not be shown. Similarly, as seen below, plague found in rats and/or fleas in these areas showed little or no apparent correlation with flea densities at the existing level. In fact plague was detected in Nakhon Sawan from *R. exulans* spleens and *X. cheopis* in April, 1953, when the total average index was only 0.6 for that city (Fig. 4).

According to Pollitzer (1954) there is reason for assuming that an *X. cheopis* index of 1.0 may be the critical index for effective plague transmission even though a positive correlation between the flea vector index and the incidence of plague does not always occur. The *X. cheopis* indices show considerable natural oscillations (Fig. 4) which might not have been so pronounced had DDT been applied in all cases as soon as the *X. cheopis* index went above 1.0.

No specific data were gathered regarding the effects of the rodenticide control program. When 1080 was used for poisoning, many dead rats were found. With Warfarin, there appeared to be a definite decrease in the number of rats obtained by live-trapping from poisoned areas.

Plague was positively detected in rats and/or fleas only three times, once in each of the 3 areas.

Headquarters	Location	Method of Detection	Collection Date	Pooled Material
Banpong	Nakhon Pathom	Bacteriological	15 Sept. 1952	*Rattus exulans* spleens
Khorat	Sisaket Uthomphon	Serological	17 Mar. 1953	*Rattus exulans* spleens and *Xenopsylla cheopis*
Nakhon Sawan	Nakhon Sawan	Serological	6 April 1953	*Rattus exulans* spleens and *Xenopsylla cheopis*

There were many instances in the Banpong area particularly, but also in the Nakhon Sawan area, where gram-negative, non-motile, bipolar staining organisms producing small pin point colonies on agar, were found, but were not tested for any of the sugars or inoculated into guinea pigs. It is felt that had all organisms been tested completely, more plague might have been detected.

Park (1941) stated that *Rattus rattus alexandrinus* (= *Rattus rattus* subsp.) and *Xenopsylla cheopis* predominated in infected villages and that *alexandrinus* formed 60 per cent of the rats collected from Bangkok. During the present study, only *X. cheopis* and its host, *Rattus exulans concolor*, were found to be agents of plague transmission. *Rattus rattus* subsp. made up less than one per cent of the total collection of house rodents in all areas, as previously mentioned, but only limited collections were obtained from Bangkok.

The field rodents, *Rattus rattus* subsp., *Bandicota indica* subsp., and *Bandicota* sp., whether trapped in the houses or in the fields near villages, were often

infested with *X. cheopis* in small numbers, as was also *R. exulans* when trapped in the fields. Similarly Traub (1950) occasionally found *X. cheopis* on field rats, *Rattus rattus sladeni* and *Rattus flavipectus yunnanensis*, in northern Burma. In deforested areas, where most human plague has occurred, fleas other than *X. cheopis* or *Ctenocephalides felis* have not been found on any animal. *X. cheopis* has been found occasionally on the ground squirrel, *Menetes berdmorei* subsp., the tree shrew, *Tupaia* sp., and the forest rat, *Rattus rajah* subsp. During the course of these studies, more than 5,000 mammals and birds and their ectoparasites were collected from the fields and forests in various areas of Thailand. Studies are under way on these collections.

Under the direction of the Thailand Division of Communicable Disease Control, the regional laboratories are continuing to operate in much the same manner as outlined in the section on methods. Although it is recognized that DDT dusting and rat poisoning campaigns are temporary measures at best, the final aim should be eradication of *Xenopsylla cheopis*, *Rattus exulans*, and plague. Furthering this aim, a lower *X. cheopis* index than 1.0 may have to be selected for initiating DDT dusting. People are being encouraged to improve sanitation by eliminating refuse in the form of rat harborage and waste food and to construct houses so as to eliminate the possibility of harboring rats in bamboo walls or thatch roofs; if the house is not elevated on posts, it is being suggested that the floor be constructed of concrete.

SUMMARY

Plague appeared in Thailand in 1904 and has been endemic ever since. Two epidemics of plague, one at the end of 1951, and the other early in 1952, prompted the Thai Department of Health to establish permanent laboratories for plague detection and control in each of the three known plague areas of Thailand. Routine procedures of control and prevention were used.

The present report discusses the work of these laboratories between February, 1952, and September, 1953, during which period a total of 48,553 domestic rats, *Rattus exulans concolor*, were obtained by live-trapping. These and some animals collected from the fields and forests were tested for the presence of plague which was detected only in *R. exulans* and its flea, *Xenopsylla cheopis*. Indices of this vector were computed monthly and an attempt was made to treat with DDT all villages, cities, or sections of cities, whenever the local *X. cheopis* index rose above 1.0. The major city in each area was studied in detail; wide oscillations were observed in the flea populations which may or may not have been due to the DDT treatment. Notes are given on the other species of rodents associated with *R. exulans*.

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FIELD TESTS OF AVIAN HOST PREFERENCE OF *CULEX TARSALIS* COQ.[1]

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Determining the host preferences of mosquitoes is difficult and complicated. A direct method is to observe to what extent potential hosts attract mosquitoes in the laboratory or field. In some studies a choice of animals has been offered to captive mosquitoes and the source of blood in fed mosquitoes is then identified by precipitin tests. Olfactometers providing a choice of air-borne attractants have been used to study a few species of mosquitoes. The most common procedure, however, has been to identify the host blood in mosquitoes found engorged under natural conditions, or to determine, by bait trapping or hand collecting, the range of mosquito species attracted to single hosts.

In studies of an encephalitis vector such as *Culex tarsalis*, a mosquito which commonly feeds on birds, some of these approaches have been of limited value. There are technical difficulties in preparing precipitin antisera that will adequately distinguish between the blood of different bird species (Hammon and Reeves, 1947). Epizootiological studies of *C. tarsalis* as a vector of avian malarias showed that this mosquito feeds on a wide range of avian species, but neither this information nor the precipitin test indicated specific host preference (Herman *et al.*, 1954; Reeves *et al.*, 1954). In a field study made in 1951, two of the authors (W.C.R. and R.E.B.) observed that when chickens were exposed individually in stable traps, the attraction and the engorgement rates of *C. tarsalis* varied in part independently. The number of *C. tarsalis* attracted to a particular chicken was frequently inversely related to the percentage which engorged, yet individual birds seemed to be constant in attracting either large or small numbers of mosquitoes and in the proportion of attracted mosquitoes which fed.

The present studies originated from the realization that knowledge of the host preferences of *C. tarsalis* might best be extended by comparing birds as attractants in bait traps. The general purpose was to determine if host preferences of *C. tarsalis* differ, first for different birds of the same species, and second for birds of different species. It could be anticipated that such knowledge would be of immediate epidemiological value in studies of the mosquito-borne viral encephalitides.

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TESTS MADE WITH ROTATED TRAPS

Types of traps used. A previously described bait trap (Bellamy and Reeves, 1952) was modified for use with birds as attractants by taking a larger lard can (of 110-pound capacity) and inserting a recess of wire screening in the top to hold a bird cage. This *screened-bird* mosquito trap (Figure 1: a and b) was subsequently redesigned so that it allowed the trapped mosquitoes to feed on the bird baits. At first the bird cage used with this *exposed-bird* mosquito trap was introduced and removed by taking off one end of the trap (the original cover), but in a still later modification (Figure 1: c) the bird cage was introduced and removed through a trap door provided with a sleeve. Bird cages made of 1″ x 1″ wire mesh were less satisfactory than those used later which were made from $\frac{1}{2}$″ x 1″ mesh.

Apparatus used to rotate traps. In all the tests reported in this section, bird-baited traps were rotated horizontally around a common axis on an apparatus known as a "rotary" (Figure 2). This device had been developed by one of us (R.P.D.), independently of studies on *C. tarsalis*, to compare insect traps and attractants. The object of rotating the items being compared is to minimize the effect of exposing traps at different sites where different population densities and other inherent factors affect the numbers of insects trapped. In the present study, the baited mosquito traps were hung about five feet above the ground, and each arm of the rotary was extended to a length of three feet. Power, supplied by an electric motor with adjustable drive, was transmitted by belt to the four-foot pulley near the base. The mechanism was usually set so that each arm made a complete revolution in about five and a half minutes.

Latin square tests and their analysis. An optional feature of the rotary test is a procedure which permits separation of the effects of baits, traps, and periods of test, and independent evaluation of each of these factors. To conduct a test accordingly, each bait is exposed in a different trap on each successive night. By shifting the baits in this manner until each has been tested in each trap, it

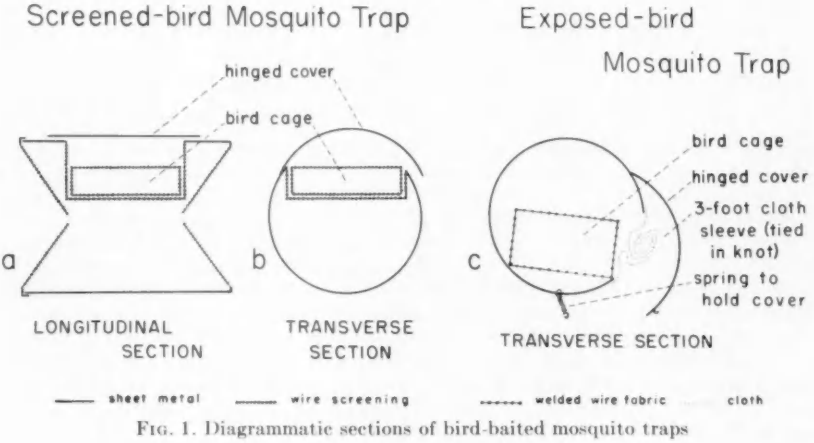

FIG. 1. Diagrammatic sections of bird-baited mosquito traps

FIG. 2. Rotary apparatus with bird-baited traps

is possible to employ an experimental design based on a randomly formed Latin square. When such a procedure is used, as in Tests 1–5, Table 1, the results can be evaluated by means of Fisher's analysis of variance (Fisher, 1947). Before making the analyses, the numbers of trapped female *C. tarsalis*, plus one, are transformed to their common logarithms, and the percentages of female *C. tarsalis* containing blood are subjected to the inverse sine transformation (Bartlett, 1947).

Conduct of rotary tests. All of the rotary tests were made in the summer and fall of 1955 near Buttonwillow, Kern County, California. The birds used for these tests were taken from outdoor aviaries to which they were returned after exposure in the field.[5] Most of the rotary tests employed four birds which were

[5] The following vertebrates were used in the rotary or other experiments reported: Brewer's blackbird (*Euphagus cyanocephalus*); tricolored blackbird (*Agelaius tricolor*); chicken (*Gallus gallus domesticus*); coot (*Fulica americana*); Chinese spotted dove (*Streptopelia c. chinensis*); mourning dove (*Zenaidura macroura*); house finch (*Carpodacus mexicanus*); red-shafted flicker (*Colaptes cafer*); pied-billed grebe (*Podilymbus podiceps*); black-headed grosbeak (*Pheucticus melanocephalus*); Western kingbird (*Tyrannus verticalis*); mockingbird (*Mimus polyglottos*); Bullock's oriole (*Icterus bullocki*); burrowing owl (*Spetyto cunicularia*); loggerhead shrike (*Lanius ludovicianus*); English sparrow (*Passer domesticus*); golden-crowned sparrow (*Zonotrichia coronata*); white-crowned sparrow (*Zonotrichia leucophrys*); barn swallow (*Hirundo eythrogastra*); turkey (*Meleagris gallopavo "domesticus"*); antelope groundsquirrel (*Citellus nelsoni*); domestic rabbit (*Orycotolagus cuniculus*); California horned toad (*Phrynosoma coronatum frontale*); Pacific gopher snake (*Pituophis cantifer*); canyon toad (*Bufo punctatus*).

TABLE 1

Attraction and engorgement of female Culex tarsalis *in rotary tests*

Test number and dates	Bird used as bait	Total *C. tarsalis* trapped in 4 nights	Per cent of *C. tarsalis* with blood*
No. 1, June 27–30	Tricolored blackbird A	50	(Test birds protected by screening.)
	Tricolored blackbird B	48	
	Tricolored blackbird C	67	
	Tricolored blackbird D	59	
		F-ratio: 1.0†	
No. 2, July 4–7	Tricolored blackbird A	65	(Test birds protected by screening.)
	Brewer's blackbird	61	
	English sparrow A	53	
	Chinese spotted dove	106	
		F-ratio: 4.8†	
No. 3, July 4–7	House finch A	169	79
	Tricolored blackbird B	194	36
	English sparrow B	227	44
	White-crowned sparrow	211	21
		F-ratio: 1.4†	F-ratio: 14.6†
No. 4, Sept. 24, 25, 26, 28	Tricolored blackbird A	215	15
	Tricolored blackbird B	269	22
	Tricolored blackbird E	251	9
	Tricolored blackbird F	239	20
		F-ratio: 1.9†	F-ratio: 4.2†
No. 5, Sept. 22, 24, 25, 26	Chicken A (1260 gm.)	272	84
	Chicken B (1130 gm.)	226	36
	Chicken C (1100 gm.)	246	82
	Chicken D (900 gm.)	217	38
		F-ratio: 4.8†	F-ratio: 16.4†
No. 6, Oct. 4–7	Chicken A	586	72
	Chicken D	434	24
	House finch A	254	60
	House finch B	260	98
	Tricolored blackbird A	181	4
	Tricolored blackbirds B, E‡	235	14
	English sparrow B	212	4
	English sparrow C	204	31

* Calculated from the four-night totals of females with and without blood.

† This value is based on bird-to-bird differences. In general an F value of 4.8 or more in a four-bait test indicates that there was a real difference among the items being compared. The analyses were made on transformations of the data for each bird on each night.

‡ Bird B was tested the first night; bird E on nights 2–4.

TABLE 2

Engorgement rates of female Culex tarsalis *attracted by carbon dioxide* (Test 7, Sept. 28)

	Chicken A	Chicken B	Chicken C	Chicken D
Weight of chicken in grams	1260	1130	1100	900
Number *C. tarsalis* caught in Test 5 (for comparison)	272	226	246	217
Per cent *C. tarsalis* with blood in Test 5 (for comparison)	84	36	82	38
Number *C. tarsalis* trapped by carbon dioxide	108	71*	92	105
Number *C. tarsalis* with blood	95	39	77	25
Per cent *C. tarsalis* with blood	88	55	84	24

* The number of *C. tarsalis* may be small in this case because of the relative condition of the dry ice container; some cartons and covers had been used previously.

tested on four separate nights. In Tests 1 to 5 the standard period of exposure was the 2½ hours following sunset. In Test 6, because of a seasonal drop in temperature, this period was changed to the whole night from sunset to sunrise. When exposed-bird traps were used (Tests 3–7), a separate count was made of the specimens of *C. tarsalis* which had fed on blood.

Results of tests: attraction. Tests did not give the simple results anticipated because host preference was found to have two major variables: the number of mosquitoes attracted and the percentage that fed on the bird (Table 1). The mosquitoes caught in the traps were all females, mostly *C. tarsalis.*

In Test 1 four tricolored blackbirds of the same size and sex attracted comparable numbers of *C. tarsalis.* In Test 2 three species of smaller birds attracted markedly fewer *C. tarsalis* than did a larger bird. In Test 3 four birds of similar size but of four different species attracted comparable numbers of *C. tarsalis.* In Test 4 four birds of the same size, sex, and species were again found to attract comparable numbers of *C. tarsalis.* In Test 5 four birds of the same species but of different weights attracted markedly different numbers of *C. tarsalis.* The numbers of trapped *C. tarsalis* paralleled the weights of these birds (White Rock chickens: A, 1260 gm.; B, 1130 gm.; C, 1100 gm.; D, 900 gm.) with minimum divergence for the two chickens of intermediate and most similar size. In Test 6 eight birds representing four species were compared simultaneously. To do this, all the trap positions on both rotaries were used interchangeably, apparently with only slight loss of uniformity. The differences in the numbers of *C. tarsalis* attracted were related more closely to the size of the bird than to any other apparent factor.

Results of tests: engorgement. In studies of host preference the final criterion of host selection is taking a blood meal. It was therefore important to learn whether engorgement accompanies attraction. Sometimes the trapped females of *C. tarsalis* took only a very little blood; but usually if they fed at all they fed to repletion. Because the total numbers of trapped specimens often differed markedly,

the percentage of females containing blood is taken as the measure of host acceptability.

In Test 3, the first in which birds were exposed to attack by mosquitoes, even though the four bird species attracted a similar number of *C. tarsalis*, engorgement rates differed from bird to bird. This lack of correlation between attraction and engorgement rates was found to hold in all later tests. Mosquitoes attracted to the immature house finch had consistently high percentages of engorgement on each of four nights (72, 80, 86, and 92 per cent). Some of the nightly rates for the other birds tested overlapped, but the overall rates ranked the male English sparrow, the male tricolored blackbird, and the white-crowned sparrow in decreasing order of acceptability below the finch.

Test 4, using four male tricolored blackbirds to compare the mosquito engorgement rates on individual birds of the same species, showed slight differences in the engorgement rates. Inconsistency in the night-to-night engorgement rates for individual birds reduced the conclusiveness of the results.

In Test 5, with four chickens previously shown to have a wide range of acceptability to *C. tarsalis* in non-rotated traps, there was night-to-night consistency both in the numbers of *C. tarsalis* attracted by each bird and in the percentages of those that engorged. Consequently, besides establishing a relation between attraction and weight of bird, the test gave unequivocal results on the relative percentages of engorged *C. tarsalis*. These differences were not directly related to the number of mosquitoes attracted, and indicated a significant difference among the four birds. To determine if these differences in acceptability were independent of the factors controlling attraction, a special one-night test was made (Test 7, Table 2). Each of four traps hung on a rotary was baited with a block of dry ice (an effective attractant for *C. tarsalis*) enclosed in two waterproofed cartons (a tapered one-pint carton inside a tapered one-quart carton). When the traps had been operated for 2½ hours starting at sunset, the entrances of the traps were closed to prevent more mosquitoes from being trapped, the dry ice containers were removed, and the four chickens were introduced. The traps were left rotating until the chickens were removed the next morning. With one exception, the engorgement rates for each bird were within the ranges of the rates observed during the four preceding nights, and the general acceptability of the four birds remained unchanged (Table 2). Thus it was confirmed that with similar trap catches of *C. tarsalis*, the percentage that will engorge on an individual host is independent of the number of mosquitoes attracted by that host, even though the latter may be related to the host's weight.

Test 6 was a comparison of four species of birds, each represented by two individuals. Whereas only the two chickens differed markedly from the other birds in attraction rates, the engorgement (acceptability) rates of the several bird species differed very widely (Table 1, Test 6). Because of the differences between individual birds, the four species tested cannot be ranked as to their acceptability except in a general and tentative way. Mosquitoes feeding on a house finch, as in Test 3, had the highest overall engorgement rate (98 per cent),

but those feeding on one of the chickens showed an engorgement rate somewhat higher than that for the second house finch.

TESTS MADE WITH TRAPS IN FIXED POSITIONS

Tests made with chickens. From July to December, 1955, observations were made in Kern County, California, on *C. tarsalis* females caught overnight in exposed-bird traps baited with chickens and suspended in fixed sites from trees. Only 13 of 54 birds were tested more than once and none more than 4 times. Since the traps were set at various sites over a long period, the range of zero to 1,565 mosquitoes captured in a trap was not surprisingly great. In 67 successful tests the engorgement rate of *C. tarsalis* ranged from 8 to 100 per cent, with a median of 70 per cent. When the numbers of trapped *C. tarsalis* are compared with the percentage of engorged mosquitoes in each catch, an inverse correlation is apparent: as the collection size increases, the percentage of mosquitoes containing blood decreases (Table 3). Despite a wide range in percentage of feeding for a given size of mosquito catch, the correlation coefficient of the relationship between number attracted and percentage feeding is $-.54$, which is highly significant at the one per cent level. Since there was evidence of the same effect in the 1951 tests made with large stable traps, the depression of engorgement does not seem to be related to the available space in the trap. Also of interest is the fact that as the percentage of *C. tarsalis* with blood decreases, the number of mosquitoes which fed includes an increasingly high percentage of mosquitoes containing merely a trace of blood.

These observations with non-rotated traps naturally raise the question whether there was similar depression of engorgement in the rotary tests. No indication of such an effect was found, presumably because the numbers of *C. tarsalis* attracted were similar and there were relatively small numbers trapped (only one separate collection of more than 200 individuals).

Tests made with various vertebrates. In a second series of observations made

TABLE 3

Relation of engorgement of female Culex tarsalis *to the total numbers caught in non-rotated traps baited with chickens*

Range in total female *C. tarsalis**	No. of catches	Per cent *C. tarsalis* with blood†	Per cent *C. tarsalis* with trace of blood in total with blood†
1–100	16	80	4
101–200	13	72	6
201–350	16	60	10
351–500	10	53	10
501–1000	11	36	13
1565	1	12	33

* Exclusive of gravid specimens and those too damaged to permit determination of state of engorgement.

† Calculated from the totals in each group of catches.

TABLE 4

Data on female Culex tarsalis *attracted to vertebrates exposed overnight in non-rotated bait traps, July 18 to 29, 1955*

Species	Number of mosquitoes		
	Total	With blood	Per cent with blood
Toad	1	0	0
Toad	0	0	0
Toad	2	0	0
Gopher snake	16	9	56
Gopher snake	14	7	50
Horned toad	0	0	0
Pied-billed grebe[1]	120	109	91
Turkey[1]	79	12	15
Coot[1]	30	15	50
Mourning dove	106	96	91
Chinese spotted dove	43	21	49
Burrowing owl[1]	43	28	65
Red-shafted flicker[2]	167	160	96
Kingbird	291	10	3
Barn swallow[1]	26	0	0
Mockingbird	78	56	72
Loggerhead shrike[2]	80	72	90
English sparrow	21	19	90
Tricolored blackbird	32	8	25
Tricolored blackbird	35	32	91
Tricolored blackbird	54	9	17
Tricolored blackbird	140	73	52
Bullock's oriole[2]	51	49	96
Brewer's blackbird	185	182	98
Black-headed grosbeak[2]	10	3	30
House finch[2]	7	6	86
House finch[2]	134	66	49
White-crowned sparrow	79	70	89
Golden-crowned sparrow	31	29	94
Antelope groundsquirrel[1]	30	0	0
Antelope groundsquirrel	31	27	87
Domestic rabbit[1]	155	1	1

[1] Test made in large stable trap, not in lard can trap.
[2] Died during test; incomplete run.
[3] Test run in daytime inside a garage.

with non-rotated bait traps (July 18 to 29, 1955), many different vertebrates were exposed overnight in one rural area in Kern County. Some of the birds were from outdoor aviaries; the rest of the vertebrates were freshly caught in the field. The traps were placed at a variety of sites, and the tests were limited in that the same animal was not exposed twice. Nevertheless the results show the wide range of vertebrates which will attract *C. tarsalis* and the great variety of animals, notably birds, on which *C. tarsalis* will feed (Table 4).

DISCUSSION

Through use of a Latin square design, rotary tests 1–5 could be evaluated with respect to differences among traps and among nights of test as well as among birds. Except in Test 2, the data on attraction showed differences among nights and not among traps. The data on engorgement failed to show any real differences among nights, and, except in Test 4, they also failed to demonstrate differences among traps. In both of the tests providing exceptions, the results with respect to birds are accordingly believed to be less conclusive.

Throughout the rotary tests made with birds, the percentage of *C. tarsalis* that engorged on a bird was clearly independent of the number caught in the trap. The size of the catch seems to measure the attractiveness of the bird, and the numbers caught are believed to be proportional to those which would reach wild birds of different attractiveness that were roosting in sites equally accessible to the mosquito.

Carbon dioxide released by dry ice, or from a cylinder of compressed gas, is a powerful attractant for *C. tarsalis* (Reeves, 1953). In respiration the amount produced by a bird is roughly proportional to its weight. Other attracting factors may be largely a combination of warmth and moisture (Peterson and Brown, 1951; Brown *et al.*, 1951). Determination of the distance from the host at which these latter factors operate must be made by future study. In the present tests it is possible that their influence was more marked in the chickens because the bodies of these much larger birds were always closer to the entrances of the traps.

The percentage of trapped specimens found engorged seems to measure specifically the acceptability of the test bird as the source of a blood meal. In nature it may represent the proportion of mosquitoes which, having been attracted to the vicinity of a bird, is likely to feed on it. Low engorgement rates may be due as much to the presence of negative or repellent influences as the lack of positive or attractive ones.

From the viewpoint of epidemiology, the total number of mosquitoes which feeds on a bird is probably the most important figure. It reflects two elements: attraction and acceptability. In some instances more mosquitoes may feed on a more acceptable bird even though it attracts comparably fewer mosquitoes. The highly attractive and acceptable bird is the individual with the greatest potential exposure to risk of infection with mosquito-borne disease agents.

SUMMARY

In comparative tests with baited traps slowly rotated about a common vertical axis, comparable numbers of *Culex tarsalis* were attracted to birds of the same size whether of the same or different species, while the number attracted to birds of different size was proportional to their size.

C. tarsalis engorgement rates were independent of attraction rates and size of bird, but they varied for different bird species and for different individuals of the same species. Engorgement rates on individual birds were usually consistent, with only a few instances of night-to-night differences. A combination of the

host's attractiveness and acceptability to *C. tarsalis* undoubtedly influences the biting attack rates on avian hosts.

A depression of the engorgement of *C. tarsalis* on chickens was found to be correlated with increased numbers of mosquitoes.

C. tarsalis is attracted to and feeds on a wide range of avian and mammalian species exposed in traps under field conditions.

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REPOPULATION CONTROL OF *ONCOMELANIA NOSOPHORA* BY MOLLUSCICIDAL APPLICATIONS AGAINST JUVENILE SNAILS THROUGH THE MEDIUM OF IRRIGATION WATER

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Successful control of *Oncomelania* snails with molluscicidal agents appears to depend on the fulfillment of two distinct requirements: (1) an initial reduction of the adult snail population to the extent that survivors are essentially isolated; and (2) countering the ability of the snail to repopulate areas despite drastic population reductions. Observations by Otori *et al.* (1954) emphasize the importance of the latter requirement. These investigators have observed that female *O. nosophora* collected from the field are capable of propagating for two years under conditions of laboratory isolation from males, and their reproductive potential seemingly is not exhausted until the third year of isolation.

In Japan where *O. nosophora* is generally associated with irrigation systems, an approximation of the first objective may be achieved by a single intensive molluscicidal spray application in the spring, when the irrigation ditches are not in use (McMullen *et al.*, 1951; Hunter *et al.*, 1952, and Moon *et al.*, 1955). An alternative procedure, currently under test by this laboratory, involves exposure of adult snails to molluscicides dispersed through the medium of temporarily established irrigation flow after need of irrigation ends in the fall (Ann. Rep., 406th Med. Gen. Lab., 1955).

Heretofore the problem of repopulation control was met only indirectly, being encompassed in the practice of making annual or biannual applications for several successive years. The possibility that repopulation could be suppressed by direct application of molluscicides against juvenile *Oncomelania* snails through the medium of flowing water was entertained in 1953 (Ann. Rep., 406th Med. Gen. Lab., 1953). The only precedent for this approach to control was the work of Kuntz and Wells (1951), who tested three chemicals as ovicides against eggs and embryos of *Bulinus* and *Biomphalaria* snails. Certain information regarding the biology of *Oncomelania* snails, as summarized by Ritchie (1955), was pertinent. The egg apparently is not an appropriate point of attack, as it is encased in a well-cemented, mud-like covering and probably deposited above the waterline. Tests revealed that the delicate, poorly-protected, newly-hatched snail is more susceptible than the egg to relatively low concentrations of molluscicides. The required rate of application is critical, since the irrigation volume-flow rate is considerable, and an unduly high concentration of chemical would make the method prohibitively costly. Since young *Oncomelania* snails are essentially aquatic for several weeks after hatching, effective exposures through the medium of irrigation water was deemed possible with applications at three-week intervals. That repopulation control would be required from early July to late August could be predicted with fair reliability on the basis of seasonal studies in Yama-

nashi. Since female *O. nosophora* can propagate for two years after isolation from males, it was evident that repopulation control would be required for at least two or three years, depending on ultimate isolation of all survivors.

Utilizing the above information, small-scale field tests were designed and conducted in two test areas during two consecutive summers. Although final evaluation will require large-scale trials, results thus far have been encouraging and tentative judgments regarding the design of the procedure have been made possible.

PROCEDURES

Following a small-scale trial in 1953, direct repopulation control with molluscicides was extended through 1954 and 1955 over two areas in the Takagawara and Funayamabashi districts of Nirasaki, Yamanashi Prefecture. In Takagawara, where molluscicides had not been used previously, 300–400 snails could be collected per man-hour, while at Funayamabashi the population had been reduced by a molluscicide from a comparable level to about 40 snails per man-hour. The first of three summer applications was planned for about the first of July, and the others at three-week intervals thereafter. Plans included an interruption of irrigation after applications to prolong the exposures. Farmers were agreeable to this except during the period immediately following rice transplanting. As a result the first application had to be delayed until mid-July.

The volume-flow rate of the irrigation water was computed by standard limnological methods. A relatively uniform 25-foot sector of the headwater ditch was marked off, the average width and depth was obtained from multiple measurements, and the time required for a dye to flow through the sector was determined. Having previously calculated the amount of chemical required for each 100 cubic feet of water per minute, it was a simple matter to equate this factor with actual volume-flow rate and siphoning time of the mixing drum in order to determine the amount of chemical required for each drum of water.

Dinitro-o-cyclohexylphenol (Dow's DN-1 formulation) was applied for six hours in a final concentration (active ingredient) of 1 ppm in 1954 and 2 ppm in 1955. Because of a shortage of the supply of DN-1, sodium pentachlorophenate had to be substituted at Funayamabashi for the second and third applications in 1955. Applications were started at daybreak to avoid the heat of the day, and after completion the irrigation flow was interrupted until the following morning, thereby prolonging exposure to about 24 hours.

Evaluation of the procedure could not be based on snail mortality or population reduction, since the procedure was designed to kill snails which were too small (0.6 to 2.0 mm. in length) to be collected in the ordinary way. The method elected was to use our knowledge of the rate of growth of *O. nosophora* and note the occurrence of young snails in the collections made immediately after the second and third applications, and again after a fourth collection made in October. According to plan, the first application was to have been made before young snails exceeded 2 mm., but enforced delay permitted growth beyond this limit in some sites at Takagawara. Nevertheless, some judgment of results was still

possible in such cases, since snails of 2.5–3.5 mm. present at the time of the first collection could be expected to attain sizes of 4.0–5.0 mm. at the time of the second collection, and unless replaced by smaller snails existing at the time of the first application effective results could be assumed.

Population samples were taken after each application on a time basis (snails per man-hour) at permanently established sites, including a control. Samples were always taken while irrigation was stopped, thus affording uniform collecting conditions throughout the experiment. The collecting sites included terminal "feeder" ditches, field edges and drainage ditches, and varied in length from one hundred to several hundred feet. It was preferable for the four to six persons in the snail collecting team to work as a unit, dividing each site into sectors and picking for a cumulative period of one hour. All snails were measured to the next 0.5 mm. with corrections for erosion. Except for a few snails with discolored shells which obviously had been dead for some time, snails in the collections were assumed to be alive and representative of the population. Extensive experience in handling *Oncomelania* has shown that most dead snails are either soon washed away or covered with silt. Critical determinations of viability were not feasible because of the length of time elapsing between collection and completion of measurements.

RESULTS

Takagawara Area

The data obtained in 1954 for the Takagawara area, where the snail population was not reduced by a previous application of molluscicide, indicated that suppression of propagation was effectively achieved in only three of six collecting sites. It was then deemed advisible to make two changes in the design of the experiment during 1955, namely, to reduce the prevailing population and increase the concentration of dinitro-o-cyclohexylphenol to 2 ppm. The former was achieved by means of a 5 ppm, 6-hour application in October, 1954. At this time irrigation had been terminated for the season, and flow was re-established only for the period of application. As noted on July 10, 1955, an overall reduction of about 70 percent had been achieved. Population reductions in the terminal "feeder" ditches were about 95 percent (sites 8, 9 and 10), while benefits were negligible at field edges (site 7) where it was impossible to raise the water more than an inch or two. Reductions of about 75 per cent were obtained in drainage ditches (12 and 13), which were reached by the waterborne chemical only after it flowed across rice fields.

In order to establish a basis for judging the results of the 1955 tests, new generation snails were classified by size into three groups, namely, 2.5 to 3.5 mm., 4.0 to 5.0 mm., and 5.5 to 6.0 mm. Snails larger than 6.0 mm. were assumed to be old ones. Since snails were measured to the next 0.5 mm., the range of the first two groups was 1.5 mm. and that of the last group was 1.0 mm. These ranges were selected since they approximate the growth that might be expected to occur during the three-week periods between applications (McMullen et al., 1951b; Sugiura

TABLE 1

Repopulation suppression of Oncomelania nosophora *with dinitro-o-cyclohexylphenol at 2 ppm.*

Takagawara, 1955

Collecting site and date	Size groupings* and number snails/man-hour				
	2.5–3.5	4.0–5.0	5.5–6.0	6.5–9.5	Total
Control					
10 July	86	151	21	369	627
2 Aug	76	117	33	339	565
23 Aug	7	41	32	407	487
20 Oct	5	23	47	484	560
Site no. 7					
10 July	3	0	0	287	290
2 Aug	2	6	0	42	50
23 Aug	1	1	7	83	92
20 Oct	0	6	2	108	116
Site no. 8					
10 July	0	0	0	4	4
2 Aug	0	4	2	2	8
23 Aug	0	2	0	2	4
20 Oct	0	0	0	1	1
Site no. 9					
10 July	0	0	0	10	10
2 Aug	0	0	0	6	6
23 Aug	0	0	0	0	0
20 Oct	0	0	0	0	0
Site no. 10					
10 July	2	0	2	14	18
2 Aug	0	0	0	34	34
23 Aug	0	0	0	8	8
20 Oct	3	1	0	72	76
Site no. 12					
10 July	44	23	1	52	120
2 Aug	6	12	15	44	77
23 Aug	0	2	6	41	49
20 Oct	1	5	1	47	54
Site no. 13					
10 July	4	19	5	117	145
2 Aug	1	8	17	122	148
23 Aug	1	1	0	89	91
20 Oct	0	4	1	52	57

* Snails were measured to the next 0.5 mm. making the range of the first two categories 1.5 mm. and the third 1.0 mm.

1933). Thus, it was expected that snails in each size group would advance to the next higher interval between collections, and overlap between new- and old-generation snails was avoided.

As recorded in Table 1, a few juvenile survivors occurred in site 7, which comprised a series of contiguous field edges formed by the stone-covered base of a dike. On the basis of the population density of 290 snails per man-hour obtained on July 10 the rate of propagation was low; furthermore, some dilution of chemical occurred here. No positive explanation is available for the variations in the adult density obtained for the several collections at site 7; similar variations were noted in 1954. The influx of snails at site 10 noted in the October 23 collection cannot be attributed to that colony. A search revealed a more probable source, namely, a field edge from which snails could have been washed. As already noted for site 7, we were relatively unsuccessful in our attempt to destroy adult snails at field edges. The sizes of the snails suggested that they were not a part of the 1955 brood hence this observation does not reflect against repopulation control but emphasizes the problem of dealing with mature snails located in rice fields. At collecting points 12 and 13 (drainage ditches) numerous young snails of 2.5–5.0 mm. were already present at the time of the first application on July 10, 1955, suggesting a good reproductive potential for survivors of the entire experimental area. Observations on numerous colonies in other areas (Ann. Rep., 406th Med. Gen. Lab., pp. 68–69, 1951) indicate that such an early appearance of young snails is the exception rather than the rule in the Yamanashi area. Subsequent to the collection on July 10, only a few snails of 2.5–3.5 mm. occurred in the collections, indicating that newly-hatched snails did not survive to replace those growing out of that size group. Furthermore, snails of 2.5–3.5 mm. and 4.0–5.0 mm. in the July 10 collection at site 13 were not equally represented in higher size ranges of subsequent collections. It appears then that dinitro-o-cyclohexylphenol at 2.0 ppm is not only highly effective against snails within the size range of 0.6 to 2.0 mm. (equivalent to 0 to 3 weeks of age), but also causes considerable mortality among 2.5 to 5.0 mm. snails, and may account for some mortality among adults as well.

Funayamabashi Area

Following a spring molluscicidal application in 1954 at Funayamabashi, the adult snail population during the following summer was about 40 per man-hour, as based on five collecting sites (Table 2). With three applications of dinitro-o-cyclohexylphenol at 1 ppm, surviving juveniles occurred at a rate of only about four per man-hour. Although this was a relatively low rate of repopulation, it was deemed advisable to increase the chemical to 2 ppm in 1955. At the time of the first application in 1955 (July 10) a decided reduction in the overall population density was evident. No molluscicides had been applied between the last treatment in 1954 and the first one in 1955; nevertheless, the population density decreased from 42 snails per man-hour in 1954 to only 6 in 1955. It is not probable that a difference this great could be due to sampling error, hence it appears that repeated exposures to low concentrations of DN-1 may have delayed effects on

TABLE 2

Repopulation suppression of Oncomelania nosophora *for two consecutive years—Funayamabashi.*

Snail size (mm.)	1954		1955	
	Second application, 4 August	Third application, 26 August	First application, 10 July	Third application, 24 August
9.5	4	7		
9.0	13	27	1	1
8.5	44	34	2	4
8.0	50	46	9	5
7.5	46	53	4	3
7.0	28	21		2
6.5	8	7		1
6.0	2	1		
5.5	4	3		
5.0	2	2		
4.5	4	4		
4.0	3	2		1
3.5	3	1		
3.0				
2.5				
Totals	212	208	16	17
Collect. time (Min)	300	300	150	245
Snails/man-hr	42	42	6	4
New gen./man-hr	4	3	0	0.25

adult snails that are manifested only after a relatively long time. If this is the case, it should greatly facilitate the whole problem of repopulation control.

At the time of the third application in 1955 (August 24), only a single young snail was found in 245 minutes' collecting time. The population residue now existing certainly consists of only isolated individuals, and if the benefits of the 1954 and 1955 treatments are equalled in 1956, virtual eradication may be attained, or at least the survivors will be devoid of reproductive potential as indicated by the observations of Otori *et al.* (1954).

DISCUSSION

Prior to this investigation, efforts to control *Oncomelania* snails with molluscides had been directed only against the adult snail by means of sprinkler applications in the spring or fall. Some indirect repopulation control may result when applications are repeated over a period of several years, since the data of Hunter *et al.* (1952) and Moon *et al.* (1955) suggest that the rate of repopulation decreases with successive treatments. For this procedure alone, however, physical conditions of the habitat, linked with the relatively long life span and prolonged fertility of females in isolation (Otori *et al.*, 1954) constitute a near-insurmountable barrier to eradication. It is now apparent that repeated application for a period of at least five years will be necessary for maximum benefits, and even

these may fall short of eradication. Evaluation and judgments are limited so far to results obtained by the researcher, and it cannot be assumed that his determined effort and efficiency will be projected into large-scale control programs. This is true of any method of schistosomiasis control at the experimental level.

The current investigation was designed to determine the feasibility of applying molluscicides against juvenile snails through the medium of irrigation water. If practicable, the procedure might serve either as a direct means of repopulation control, subsequent to control measures applied against the adult snails; or if conjoined with natural mortalities among adults, the prevailing population should ultimately be exhausted. The latter does not appear feasible because of the relatively long life span of *Oncomelania* snails; this was apparent from the results at collecting site 7 in Takagawara. Propagation was effectively suppressed in both 1954 and 1955, while a specific attempt to reduce the adult population in the fall of 1954 was ineffective. Although there has been a gradual reduction in adults in this site, it appears that at least three years, if not four, might be required before survivors would be in a state of isolation; and further time probably would be required to exhaust the reproductive potential of surviving females. This assumption is consistent with the observations on the life span of *O. nosophora* by Sugiura (1933) who noted that painted snails could be recovered in numbers after three years, but were reduced to a minimum after four years; also eggs were obtained from one four-year survivor. It is apparent, then, that reduction of the prevailing population and repopulation control should be considered complementary endeavors.

A single preliminary application of chemical to reduce the prevailing snail population appears adequate, even though the initial objective of isolation is not achieved. Supplementary benefits, as indicated from the tests at Funayamabashi, can also be expected from repopulation control. The reduced population of 42 snails per man-hour of collecting existent at the onset of the test was in excess of isolation; however, this figure was reduced to 6 at the end of the first year. Virtual isolation of all survivors by the end of the first year of control might well be designated as an arbitrary goal, and whether it can be attained with a residue greater than 40 snails per man-hour should be determined.

Dinitro-o-cyclohexylphenol at 2 ppm appears to be near-optimal for repopulation control. Results at Takagawara (Table 1) suggest that while snails of all sizes are susceptible to this concentration to some extent, its effectiveness is very high among snails of 3.5 mm. or less, approaching 100 percent among those under 2.5 mm. in length. The possibility that 1 ppm may prove adequate after the second summer should be considered. Three summer applications at three week intervals appear to have been judicious, although two applications at four-week intervals might prove adequate after the second year. The date of the first application may tentatively be set for mid-July in Yamanashi, but can best be determined by preliminary survey of conditions related to rice culture and the time when juvenile snails begin to attain a growth of 2 mm. In any sizeable area the latter time will vary for different sites, and is probably dependent on the diversity of habitat. Where uninterrupted wetness prevails in advance of full-

scale irrigation, early propagation may be anticipated. The number of years required for repopulation control remains uncertain, but results at Funayama-bashi indicate that virtual eradication may possibly be achieved after three years. In order to achieve this end it is apparent that some supplementary control procedure, such as spraying or introducing chemical into impounded waters at limited points, will be needed.

Evaluation of the procedure for repopulation control described herein is complicated by two distinct problems. First, the procedure is directed against snails too small to be collected by usual methods. This problem was resolved indirectly by determining fluctuations in the occurrence of new-generation snails in collections made immediately after the second and third summer applications, and again in the fall. The second problem lay in the selection of a suitable control area. The theoretically perfect control area should meet certain requirements. It should encompass a section of an irrigation system large enough to be representative of all the varied habitat conditions to be found in the experimental area. In this way allowances could be made for variations in rate of reproduction in different colonies, and assure a normal control baseline. In addition, the control area probably should also receive the initial application of molluscicide to reduce the adult snail population. The control area of Takagawara was not entirely adequate in these respects; nevertheless, observations made in the present study cannot be invalidated by this alone. Female snails collected at Takagawara from the control site at the time of the first and second applications in 1955 produced many offspring in the laboratory, suggesting that potentialities for repopulation did exist. Furthermore, results of field tests by Hunter *et al.*, (1952) and Moon *et al.*, (1955) show that repopulation occurs rapidly in a variety of situations and thereby give assurance that the low levels of repopulation achieved in the present investigation are attributable to the chemical treatment.

The relative cost of snail control by the plan here reported as compared with the spray method previously employed has received only cursory consideration. Comparison is hardly warranted until several matters are clarified, namely, the number of applications necessary each summer, the number of years required to achieve maximum benefits, the cost-efficiency index of the ultimate chemical of choice, and whether it will be necessary to allow the waterborne chemical to enter rice fields or whether exposures can be limited to irrigation channels. Direct repopulation control may increase costs, but not prohibitively so, if the applications are not required beyond the limit of three years.

SUMMARY

Two distinct requirements for successful chemical control of *Oncomelania nosphora* should be recognized, namely, reduction of the prevailing population to a level that would result in isolation of the survivors; and repopulation control directed against the juvenile snails. These two requirements are considered complementary for ultimate success with molluscicides. An experiment, primarily an evaluation of a procedure, has been carried out which entailed three 6-hour applications of dinitro-o-cyclohexylphenol (1 or 2 ppm) in irrigation water at

3-week intervals during the summer. Irrigation flow was interrupted for 18 hours after each application, during which time snail collections were obtained on a time basis (snails per man-hour). Evaluation was based on reduction of the juvenile snail population. Results indicated that repopulation control directed against juvenile *O. nosophora* is feasible in the irrigation system of Japan if initial reduction of the prevailing adult population is carried out.

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STUDIES ON THE QUALITY OF FRESH WATERS OF PUERTO RICO RELATIVE TO THE OCCURRENCE OF *AUSTRALORBIS GLABRATUS* (SAY)

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Several field studies have suggested that the quality of water is important in determining the distribution of the snails which are vectors of schistosomiasis in the new world (Sioli, 1953; Scott, 1942; Luttermoser *et al.*, 1945 Harry *et al.*, 1956a, 1956b). Because of the many factors involved in the concept "water quality", which must be considered in relation to the life history of the intermediate host and the several stages of the parasite associated with it, a complete understanding of them is likely to be long delayed. The present paper reports the observed range of variation in certain environmental factors in some of the fresh waters of Puerto Rico. These observations may help in formulating a more critical definition of the natural habitat of the snail, and in developing experimental procedures which more nearly simulate natural conditions. Data were collected over a two-year period in conjunction with other field studies (Harry *et al.*, 1956a, 1956b).

METHODS

More than 130 extensive water analyses were made and included most of the factors discussed below. Studies were repeated at several stations to determine the variability of these factors. Many additional determinations were made of those factors in which field analyses were feasible.

Alkalinity, temperature and pH (using La Motte liquid color standards for the last) were determined in the field. Calcium and magnesium were determined in the first 50 samples by the gravimetric and colorimetric methods, respectively, recommended by The American Public Health Association (1946). Later the method of Diehl *et al.* (1950) was used to determine total hardness, and that of Betz *et al.* (1950) was used for calcium hardness. Magnesium hardness was then computed by obtaining the difference. A Type RC Conductivity Bridge (Industrial Instruments Co.) was used to determine conductance. All other analyses were by the procedures recommended by The American Public Health Association (1946). Bottom samples from the larger reservoirs were obtained by a Modified Kemmerer water sampler (Ellis *et al.*, 1948).

RESULTS

Temperature. The total range of water temperatures recorded was 21°–35°C. Small, high gradient streams at higher elevations and springs at any elevation generally had low temperatures, regardless of the time of day or season. *Austra-*

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lorbis glabratus does not occur in either type of habitat, though perhaps for other reasons than temperature.

Many habitats of this snail varied widely within the recorded range of temperature. A limestone sink pond showed a range of 23°–35°C. in readings taken on 15 different days extending over a two-year period. The lower temperatures, 23°–27°C., all occurred in the mornings, and the highest, 35°C., was recorded on four occasions in the middle or late afternoon. Both maximum and minimum temperatures were recorded during winter and summer months. This pond tended to show a marked diurnal variation, but no seasonal cycle was evident in the limited data. Temperatures of 35°C. have been found in other limestone sink ponds and alluvial marshes in the afternoon. In such situations, due to the dense vegetation and the attendant poor circulation of water, a decreasing temperature gradient of as much as 3°–4°C. may be found in the upper foot of water.

Stream habitats of *A. glabratus* similarly showed fluctuations of temperature of about the same range. Eighteen temperatures were taken at intervals of two- to three-weeks over a period of more than a year on a small stream near San Juan. These ranged from 22°–31°C. The times of sampling were about equally divided between morning and afternoon, and no regular seasonal or diurnal cycle of temperature was evident. Sixteen of the temperatures were within the range of 26°–28°C., a range shown experimentally by Stirewalt (1954) and DeWitt (1955) to be nearly optimum for penetration, development and longevity of *Schistosoma mansoni* in *A. glabratus*. This stream frequently contained infected snails.

The surface temperatures of several larger reservoirs (Dos Bocas, Guineo, Cidra, Loiza, Curias, Caonillas) showed a range of only 26°–30°C. when sampled from September through November, 1953. The range was not exceeded by resampling several of these and additional reservoirs in March and April, 1954. The greatest difference between surface and subsurface temperatures was found in Caonillas Reservoir in November, 1953: 29°C. at the surface and 25°C. at 34 meters depth. Temperature can scarcely be the factor which excludes established colonies of *A. glabratus* from these reservoirs.

The snail can be kept for several days or a week at 15°C. There were fewer mortalities at this temperature than at room temperature (25°–32°C.), especially when the snails were crowded in small containers, as was often necessary when material was brought in from the field for quantitative studies on snail populations and incidence of infection. Temperatures as low as 6°C. usually produced high mortalities under comparable crowded conditions.

Electrolytic conductance and dissolved solids. It was not feasible to control temperature on most of the conductance readings. A total range of conductance of 87 to 7142 $\times$ 10^{-6} mho, within the range of temperature of 25°–32°C., was found in fresh waters of the island. The dissolved solids of the same samples, as determined by drying, ranged from 69 to 3100 ppm. Despite the failure to control temperature in making the conductance analyses, there was a general correlation between the conductance at temperatures noted and the dissolved solids, so that it was possible to estimate the latter from the former.

To determine the degree of error which lack of temperature control introduced,

the conductance of 69 samples of water was determined at room temperature 25°–32°C. and after cooling the samples to 25°C. The samples were randomly chosen, but included a large variety of habitat types and range of dissolved solids. A scatter diagram plotting the specific resistance (in ohms) at 25°C. and per cent change per degree above 25°C. for each sample showed there was no correlation between the per cent change per degree increase in temperature and the magnitude of the specific resistance at 25°C. There was a general tendency for waters in the range of 26°–28°C. to have a greater per cent of change of resistance per degree C. than there was in waters in the range of 29°–32°C. The per cent change in resistance per degree above 25°C. varies in different waters, and may not be constant for each degree above 25°C. in a given water. The average per cent change in resistance per degree was 2.6, with a range from 0.37 to 5.86.

Most waters which have less than 150 ppm. dissolved solids, represented by a conductance of about 222×10^{-6} mho at the temperatures cited, are free of established colonies of *A. glabratus*. Such waters were found in three types of aquatic situations: (1) small temporary pools, (2) major reservoirs of the Oldland Physiographic Province (Harry *et al.*, 1956a) and (3) some streams, particularly those with long headwater reaches of higher gradient.

Temporary pools may have a conductance of 100×10^{-6} mho. These often contain *Tropicorbis decipiens*, a small planorbid snail rarely present in other types of situations and never found as established populations with those of *A. glabratus*.

The major reservoirs of the Oldland Physiographic Province (Loiza, Dos Bocas, Guineo, Cidra, Curias, Caonillas, Patillas) had a conductance ranging from 222×10^{-6} mho (Loiza) to 87×10^{-6} mho (Guineo). This represented a dissolved solid content of 159–69 ppm., respectively. A variety of mollusks occurred in one or more of these reservoirs, including *Doryssa* sp., *Ponacea* sp., *Helisoma* sp., *Physa cubensis*, *Lymnaea columella*, *Uncancylus concentricus*, *Tropicorbis albicans* and *T. riisei*. However, *A. glabratus* was found only as occasional individuals in the headwater coves of the Loiza and Cidra Reservoirs. Both of these lakes have headwater streams maintaining the snail.

The water of the Guajataca Reservoir, which is situated entirely on limestone, had a conductance of 294×10^{-6} mho, and 169 ppm. dissolved solids. No mollusks were found in this lake. As far as could be determined by our analyses, the quality of water in the aqueduct below the dam and in the reservoir was approximately the same. However, *A. glabratus* as well as *Physa* sp., *Tropicorbis riisei* and *Lymnaea cubensis* occurred in the lower gradient reaches of the aqueduct. The reason for this discrepancy is not known, but it suggests that something other than the amount of dissolved solids *per se* is the limiting factor. *A. glabratus* also occurs abundantly in the aqueduct leading from the Patillas Reservoir, yet it is not established in that lake. The concentration of dissolved solids of the reservoirs, away from their headwater coves, is generally lower than those in the streams at a point just above their entrance to the reservoirs, when the streams are at normal low flow stage.

At a given point along a stream, the concentration of dissolved solids was ap-

proximately constant when the stream was at normal low stage. Samples from a series of stations along 11 streams showed that in those having marked differences in gradient between the headwater and lower reaches, the concentration of dissolved solids increased in the downstream direction. Examples of such streams are the Canovanas, Patillas and other rivers at the east end of the island (see Harry and Cumbie, 1956b, for gradient profiles of these streams). Upstream stations may have less than 150 ppm. dissolved solids at normal low stages, and thus are more dilute than most waters in which *A. glabratus* occurs. During flushing, the dissolved solids at a given point in the stream decrease. The decrease is greater in the upstream direction. The interval of time, in which the water at a particular point may have smaller amounts of dissolved solids than is presumed favorable for *A. glabratus*, may be an important factor in the upstream limit of the snail. Although the greater current velocity of steeper segments of a stream probably plays a part in the upstream limits of the snail, disjunct marginal pools may contain the snail much farther upstream than the upper limit of the population in the main channel. Such pools generally have a higher dissolved solid content than the adjacent stream channel at normal low stages, possibly due to the effect of evaporation on the pools.

No appreciable downstream gradient of dissolved solids was found in streams of very low slope, such as those of the Sabana Llana type near San Juan (Harry and Cumbie, 1956b). In these streams the dissolved solids are well above the proposed critical minimum except at times of more extreme flushing.

Three streams are known in which *A. glabratus* colonies exist in waters of less than 222×10^{-6} mho conductance. The Loiza River at San Lorenzo had a conductance ranging from 142 to 166×10^{-6} mho (12 samples) at normal low stages. The dissolved solids of five samples ranged from 126 – 237 ppm., but averaged 170 ppm. This may be a case in which the conductance does not correlate well with the amount of dissolved solids (Ellis *et al.*, 1948). The two other cases are the Patillas River well above the Patillas Reservoir (station 143, Harry and Cumbie, 1956b) and the Santiago River above Naguabo. Here again the conductance may not represent the dissolved solids very closely, but conclusive data are lacking. All three streams have long, high-gradient headwater reaches, and the headwater areas of the Patillas and Loiza are adjacent.

At normal low stages, streams on limestone had more than 150 ppm. dissolved solids, as might be expected from the soluble nature of the substrate. However, *A. glabratus* had not been found in them. Beyond their temporary headwater reaches most are of moderate to low slope, and many long segments have a slope as gentle as streams of the Oldland and Alluvial Provinces which contain the snail (Harry and Cumbie, 1956b). Limestone sink ponds and marshes on alluvium generally had more than 150 ppm. dissolved solids. Surface samples contained less immediately after heavy rains but this is probably a phenomenon of brief duration.

Habitats which are optimum for *A. glabratus*, on the basis of persistence of population density and size of the adult snails, generally had a dissolved solids content ranging from 170 to 600 ppm. The snail had been found in waters of about

3,000 ppm. dissolved solids in a limestone sink pond near the sea. Pollution increases the dissolved solids, and this effect remains even beyond the recovery zones of a stream in which the organic content had been oxidized.

Some situations have physical characteristics and biotic assemblages which elsewhere are associated with *A. glabratus*, yet they lack this snail. The dissolved solids and all individual components for which analyses have been made may be well within the range favorable to the snail. In the case of isolated sink ponds on limestone, it may be that snails have never been introduced. However, for streams like the Arroyata and Guadiana (Harry and Cumbie, 1956b), which empty into the snail-infested La Plata River, some other explanation must be sought.

Perhaps some more specific factor than dissolved solids *per se* is associated with the general absence of *A. glabratus* in dilute waters. In one experiment, adult snails were maintained in redistilled water for two weeks, without any apparent harmful effects on them. Fewer mortalities occurred in the test lot (20 snails per liter of water) than in the control, which was kept in tap water. Lettuce was the only food provided. The water was changed almost daily to insure a constant low level of dissolved solids in the test lot. Eggs were produced in both lots. Those in the control hatched in about eight days, while those in the experimental lot, although apparently developing at the normal rate, seemed to have difficulty in escaping from the gelatinous egg mass. Very few juveniles emerged. While it is evident that the adults can withstand waters having little dissolved solids for short-term experiments, there is a suggestion that such water may be unfavorable for eggs or juveniles.

Hydrogen ion concentration. A pH range of 6.0 to 9.1 was found, but surface waters beyond 7.0 to 8.4 were rare. Waters below pH 7.0 occurred chiefly in marshes, particularly those present on alluvium derived from granite areas, and in some limestone sink ponds. However, waters in both the granite areas and sink ponds ranged upward, the former to pH 7.6 or 7.8, and the limestone sink ponds even higher. A pH of 9.1 was found in Lake Tortuguero, which did not contain the snail, and in Lake Cartagena, which had a few snails in the marginal vegetation. While the snail had been found throughout the total range of pH encountered on the island, abundant populations of *A. glabratus* tend to occur in habitats with a pH of 7.0 to 7.6.

Within a given habitat the pH may vary widely. The Dona Ana Creek near San Juan ranged from pH 6.7 to 8.1 during a two-year period. Five of the 18 samples had a pH of 7.0, and most of the other samples were alkaline but near neutrality. Ten samples of a limestone sink pond containing the snail fluctuated between 7.1 and 8.6 during a period of 18 months. No apparent correlation was found with season, time of day or similar factors. Periodic data on several other bodies of water support the conclusion that the pH may vary widely in a given habitat within a matter of days.

Major inorganic constituents. Table 1 shows that *A. glabratus* occurred in the higher concentrations of the major inorganic components considered. Excepting sulfates, the lower concentrations of these ions were usually in waters of low

TABLE 1

The range of A. glabratus *relative to the total range in ppm. of the major inorganic ions for which analyses were made*

Ions	Total range		Range of *A. glabratus*		Range optimum habitats of *A. glabratus*	
	Min.	Max.	Min.	Max.	Min.	Max.
HCO_3 and CO_3	20	415	45	415	70	120
Cl	5	2150	20	2150	20	110
SO_4	2	133	2	133	10	80
Ca	12	129	17	129	17	70
Mg	1	103	5	103	5	50

total dissolved solids. The values given under the column "optimum habitats" represent a compilation of several streams of the alluvial areas and limestone sink ponds in which the snail was abundant and persistent.

Australorbis habitats having higher total alkalinity (bicarbonates and carbonates) were in the recovery zones of polluted streams (340 ppm. HCO_3 in the Santo Domingo Creek at Cayey) and in limestone sink ponds near the coast (365 ppm. HCO_3, 50 ppm. CO_3, pH 8.3). Usually the normal carbonates constituted less than 20 per cent of the total alkalinity. Lake Cartagena was exceptional, with 90 ppm. CO_3 and 35 ppm. HCO_3 (pH 9.1).

Except in the streams flowing entirely on limestone, the higher total alkalinity values were accompanied by higher values of chloride ions. In the recovery zone of the polluted Santo Domingo Creek at Cayey, 110 ppm. Cl was found. Many of the *Australorbis* habitats near the coast contained one hundred to several hundred ppm. Cl, and the maximum found, 2,150 ppm. Cl, occurred in a limestone sink near Arecibo.

The amount of sulfate present in the water of Puerto Rico seems to be independent of proximity to the sea, pollution, or other ions present. Sulfates tend to be low (less than 20 ppm.) in streams of the granite areas even when the total dissolved solids are more than 150 ppm.

Limestone streams and those on alluvium adjacent to limestone had the highest concentrations of calcium (100 to 129 ppm.). Limestone sink ponds vary in the amount of calcium they contain, and some of the lower values were from such situations. This is because the waters of those ponds become more or less insulated from the underlying bedrock by noncalcareous silts. On the other hand, two examinations made at the Guajataca Reservoir, entirely within the Tertiary Limestone of the north coastal area and surely exposed to much bedrock, showed only 42 and 47 ppm. calcium. The precipitation of calcium in the fresh waters of Puerto Rico is rare. Occasional small deposits were found in some streams on limestone. Lake Tortuguero, a large remnant of a former marine lagoon, is unique in having a fine, white, calcareous precipitate which renders the water turbid.

In most waters the magnesium content is less than half that of calcium. However, in the serpentine area and some coastal regions, particularly the arid southwestern corner of the island, magnesium may exceed calcium. No correlation

TABLE 2

Stations in which copper exceeded the concentrations in which A. glabratus *was found. Dissolved solids (TDS) and Cu in ppm.*

Station	TDS	Conductance	Cu	Locality
4.1	114	192 × 10^{-6} mho	0.20	Cibuco R. in Oldland Province
223.1 S	120	142	0.26	Guayanes R. (Yabucoa) in Granite Area
223.1 M	103	113	0.33	Marshy pool by 223.1 S
207.1 T	121	166	0.20	Cidra Reserv., top
207.1 B	149	181	0.20	Cidra Reserv. at 30 ft. depth
209.1	402	666	0.23	Stream near Juncos
209.2	357	606	0.20	Stream near Juncos

was found between the calcium-magnesium ratio and the distribution of *A. glabratus*.

Copper. Because a number of small but high-grade copper deposits are known about the island, and since this element has been used as a molluscicide, a few analyses were made to determine whether it might be a natural limiting factor on the distribution of the snail. Analyses of 56 natural waters showed a range of 0.000 to 0.330 ppm. copper. Habitats containing *A. glabratus* ranged from 0.000 to 0.180 ppm. All of the latter had more than 150 ppm. dissolved solids, as noted earlier. The snail was not found in concentrations of copper that ranged from 0.200 to 0.330 ppm. Analyses of these samples, shown in Table 2, suggest that the higher copper concentrations occurred in waters of low dissolved solids. The two exceptions (station 209) were both from a small stream whose temporary headwaters drain a small iron mine. Some ore from this mine assayed more than 10 per cent copper (verbal communication, Dr. Ray Smith, formerly of the U. S. Geological Survey in Puerto Rico). Not all natural water which was low in dissolved solids had a high copper content.

In laboratory experiments it was found that 1.0 ppm. or more copper (in copper sulfate) in both distilled water and waters which contained more than 150 ppm. dissolved solids generally caused the snail to retract far into the shell, bleed and die within 24 hours. A different reaction was produced in short-term experiments by much smaller concentrations of copper in waters having low concentrations of dissolved solids. Concentrations of 0.050 to 0.100 ppm. copper added to distilled water caused the snails to extend without attaching to the substrate. The tentacles usually contracted, the base became bulbous, and the snails exhibited feeble motion that suggested distress. After 24 hours there was lessened ability to retract into the shell when touched. If transferred to waters within the optimal range of dissolved solids and low in copper, the snails generally resumed normal behavior after a few hours. The tentacles again became long and filiform, showing no gross evidence of damage from the experiment. In short-term experiments no gross effect was produced in snails exposed to concentrations of less than 0.050 ppm. copper in distilled water.

The tonic effect was also eliminated by bubbling hydrogen sulfide gas through distilled water containing deleterious amounts of copper, if it was filtered to re-

move the precipitate and aerated to remove excess gas. The resulting precipitate of sulfur seemed to have no effect on the snail. Detoxification was also achieved by adding an excess of calcium hydroxide to distilled water containing 0.090 ppm. copper, then saturating the solution with carbon dioxide. Analysis of such water after filtration showed only 0.015 ppm. copper remaining in solution.

Additional laboratory experiments have shown that other metals introduced into distilled water, particularly cadmium and zinc, produce the same effect on *A. glabratus* as copper, and at almost the same concentrations.

DISCUSSION

The water temperatures of Puerto Rico are very similar to those reported from other parts of the range of *A. glabratus*. In Venezuela, Luttermoser *et al.* (1953) found the snail at temperatures of 18°–30°C., and Deschiens *et al.* (1953) reported the same range for habitats of this snail in Guadeloupe during January and February. Lower temperatures within this range may not affect the snail appreciably, but Stirewalt (1954) and DeWitt (1955) showed that temperatures in the range of 22°–25°C. were unfavorable for the development of *S. mansoni* in the snail. Lower temperatures might possibly be a limiting factor in the geographic distribution of *S. mansoni*, if not on the distribution of the snail. While the snails occasionally experience temperatures of 33°–35°C. in nature, these probably are of only a few hours duration. Stirewalt (1954) and DeWitt (1955) found that high mortalities resulted in snail cultures maintained at constant temperatures above 30°C.

Determination of the concentration of dissolved solids furnishes a useful index to the general conditions of an aquatic enviroment. Within the narrow range above 25°C. encountered in Puerto Rico, conductance readings indicate concentrations of dissolved solids that are sufficiently accurate for most practical purposes. The average change of specific resistance per degree C. found in the present study, 2.6 per cent, agrees closely with the 2.0 per cent per degree recommended as a correction factor by Ellis *et al.* (1948) and Gustafson *et al.* (1939).

From Table 1, it is evident that the higher concentrations of bicarbonate, carbonate, sulfate, chloride, magnesium and calcium ions which occur in natural fresh waters do not exclude *A. glabratus*. The experimental data of Deschiens (1954) shows that the lethal concentrations of these major ions is far above those which occur in Puerto Rico. The ability of the adult snail, at least, to live in redistilled water for periods of weeks (as long as the experiment was continued), and the occurrence of snails in some waters having low amounts of dissolved solids (Loiza, Patillas and Santiago Rivers) suggests that some other factor than the scarcity of these ions is responsible for the absence of the snail in the great majority of waters low in total dissolved solids.

The analyses of waters of the Amazon region given by Sioli (1953) show that the amounts of materials dissolved in these waters are uniformly much lower than waters harboring *A. glabratus* in Puerto Rico. This snail, although known from other parts of Brazil, is not among the five species of Planorbidae which Sioli reported from those waters. Other species of snails, including Planorbidae,

live in waters having low amounts of dissolved solids in Puerto Rico, as pointed out above in the case of major reservoirs and temporary puddles. Both Sioli (1953) and Deschiens (1954) cite other species of schistosome vectors from waters of very low dissolved solid content.

Studies on the copper content of natural waters indicate that this metal, and perhaps others, might be a limiting agent in waters low in total dissolved solids. In waters with high concentrations of dissolved solids, concentrations of heavy metal ions might be expected to be low, due to the increased amounts of carbonate or bicarbonate and possibly in some special cases to the presence of hydrogen sulfide.

The question of calcium, both in excess and deficiency, as a limiting factor in the presence of fresh water snails has long been considered, without conclusive results (Frömming, 1938). In the present study it was noted that *A. glabratus* is absent from streams on limestone, in which calcium and carbonate or bicarbonate are high, yet it is present in limestone sink ponds, drainage ditches and marshes near the sea, where these radicals are also high. Perhaps attention should be directed to the weak acids (carbonate and bicarbonate) rather than to the alkaline earths (calcium and magnesium). In waters where *A. glabratus* was present, irrespective of the absolute amount of the individual radicals, the weak acids were usually one to three times as abundant as the strong acids (chloride and sulfate) in equivalents per million. However, the weak acids were four to six times greater than the strong acids in the streams on limestone. Possibly the ratio between weak acids and strong acids is a significant factor in limiting the distribution of this snail. Circumstantial evidence in support of this hypothesis is found in the widespread practice of introducing calcium sulfate into laboratory cultures of snails for its beneficial effects, and the occasional use of lime as a molluscicide (Buckley, 1946). The former decreases the strong acid radicals, and the latter those of the weak acids.

Hydrogen ion concentration does not seem to be, *per se*, a limiting factor in the distribution of the snail. Deschiens (1954) found that *A. glabratus* tolerated experimentally a range of pH 4.5 to 10.0. The range of pH found in Puerto Rico agrees closely with that report for *A. glabratus* habitats in Venezuela by Luttermoser *et al.* (1945) and that in Guadeloupe reported by Deschiens *et al.* (1953).

SUMMARY

In Puerto Rico, *Australorbis glabratus* occurs throughout the range of pH and temperature found. It occurs in the maximum concentrations of CO_3, HCO_3, Cl, SO_4, Ca and Mg found in fresh waters here. It is generally but not invariably absent from situations normally having less than 150 ppm. dissolved solids. Yet experimentally, the snail can be maintained for at least two weeks in redistilled water. Small amounts (about 0.1 ppm) of copper in distilled water produce distinct, incapacitating distress in the snails within a few hours time. Copper concentrations in natural waters range from 0.000 to 0.330 ppm. Most concentrations of copper greater than 0.020 occur in waters of less than 150 ppm. It is suggested that copper, and perhaps other heavy metals, may be the factors responsible for

the absence of this snail in most dilute waters. A large excess of weak acid radicals (CO_3 and HCO_3) over strong acid radicals (Cl and SO_4) is suggested as related to the absence of this snail in flowing waters on limestone.

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THE TREATMENT OF SNAKE BITE

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Although the number of deaths due to snake bite in the world is estimated, by Swaroop and Grab (1954) to be between 30,000 and 40,000 annually, death from snake bit is not a major medical problem in the United States. In Arizona, where sixteen species of rattlesnake and the coral snake are found, only twenty deaths occurred during a twenty-six year period (Stahnke 1956b). Wood (1954) in his report of 200 cases of snake bite occurring in Virginia reported no fatalities. Among 2,376 cases from throughout the United States, Githens (1935) found 128 deaths. In cases where they did not receive antivenin, 14 per cent resulted in death but among those receiving antivenin the fatalities were reduced to 3.7 per cent. Swartzwelder (1950), reporting on 306 snake bite accidents in Louisiana, states, "the last recorded fatality associated with snake bite occurred in 1921 . . . no deaths resulting from snake bite have been recorded for at least 25 years in this hospital." Swaroop and Grab (1954) state, "we may presume that perhaps only about 10 to 20 deaths from snake bite occur each year in the U. S. A.[4] If Arizona is an indication of a trend, this figure will be very much reduced since there were no deaths here from snake bite in 1954 and 1955.

Almost all venomous bites in the United States are inflicted by pit-vipers, i.e., rattlesnakes, copperheads, and cotton-mouth moccasins. This is due to their abundance and the fact that their habits and habitats conflict with the activities of man. The proteolytic, hemorrhagic, hemolytic and anti-bactericidal reactions of these venoms, which have not been receiving adequate attention, caused considerable difficulty. Although not lethal per se, they cause tissue destruction of such proportions that, in a high percentage of cases, the result is either dysfunction or complete loss of a finger, hand, arm or other limb. When the intense suffering, economic loss in man-hours and medical care is added to this, a problem of real dimensions is revealed. What is more, the size of the snake is not the controlling factor as it is in the problem of death. Serious tissue destruction can result from the bite of even the youngest of pit-vipers (Stadelman 1928).

The most commonly recommended treatment for snake bite, i.e., the tourniquet, multiple incision, and suction (popularly known as the tourniquet, cut and suction method) does very little to alleviate the problem of tissue destruction, and does in fact aggravate it. In the hands of the layman the T.C.S. technique is generally very destructive and even in the hands of the most skilled, this treatment gives the average physician no choice but to let the enzymatic action take

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[4] Our experience indicates that some of the deaths credited in vital statistics to snake venom are apparently due to secondary effects arising as a result of poor management.

its course and hope that the tissue destruction will not be too great before the enzymes are neutralized through natural processes.

The following case, reported by one of us (Tenery and Koefoot 1955), illustrates what may be expected when the customary procedure is used. A 23-year-old

FIG. 1. Appearance of hand and forearm four days after being bitten by rattlesnake. The large hemorrhagic blebs and edema are apparent.

FIG. 2. Two weeks following the bite there is extensive necrosis of the dorsum of the hand and thumb. The paucity of drainage belied the fact that this was a deep and widespread necrotic process.

FIG. 3. Five weeks following the bite debridement has removed most of the necrotic muscle and bone. The dorsum of the hand has a provisional skin graft in place. Further necrosis in the first intermetacarpal space is occurring and the thumb flexor is visible in the depths of the space. Its removal was necessary.

FIG. 4. Adequacy of thumb abduction and revision of thumb remains to provide a tactile stump end are shown.

amateur herpetologist sustained a bite on the dorsum of the right thumb web by a four-foot eastern diamondback, *Crotalus adamanteus*. A cloth tourniquet was applied to the forearm and incisions were made through the fang marks. Although shallow, they produced a "good" flow of blood on oral aspiration.

Within a half hour after the bite, he was hospitalized and antivenin administered in the prescribed manner. The right arm, which had during the next two hours become tensely swollen, developed a massive brawny edema with hemor-

rhagic bleb formation (Fig. 1). By the fifteenth hour after the bite he appeared moribund despite an additional 30 ml. of antivenin and 500 ml. of whole blood. At this point, ankle veins were cannulated and over a period of several hours 1,500 ml. of whole blood containing 45 ml. of antivenin was administered. The patient rallied during the following six hours and on the day following the bite, the blood pressure, and hematocrit reading had risen to normal levels. On the ninth day a debridement of the necrotic skin was carried out but the resultant open wound failed to exhibit healing tendencies (Fig. 2). After a number of dressings with no apparent improvement the patient was transferred to a hand surgery center one month following the bite, and in the five weeks following the bite, debridement had removed most of the necrotic muscle and bone (Fig. 3). The raw area was now covered with a split-thickness graft all of which survived. Further necrosis necessitated a second debridement before the last small pocket of the wound was healed in the eighth week following the original trauma. The reconstruction of this hand required the deletion of the index finger and transfer of an abdominal pedicle and occupied the following eight months (Fig. 4). The resulting deformity amounted to loss of the index finger and a short, practically immobile thumb. The patient was discharged ten months after his original injury.

A close parallel to the above case, but one in which cryotherapy was used, illustrates what can be done to prevent both death and excessive tissue destruction. The victim, a laboratory assistant accustomed to handling venomous reptiles and acquainted with cryotherapy, was a 26-year white male weighing 175 lbs., in excellent general health. The snake was a 3½ foot *Crotalus atrox*.[5] The bite was inflicted on the terminal phalanx of the right thumb with the fang penetration in the fat pad of the thumb, the left fang making a deep puncture wound and the right fang making a slashing wound. Immediately following the bite, a shoe string was wrapped tightly about the base of the thumb, twisted with a hemostat, and the hand and forearm immersed in a five gallon can filled with ice and water. After approximately nine minutes, while the patient was being transported to the hospital, the ligature was removed. Neither incision and suction nor antivenin were employed. At the hospital, cryotherapy was continued; the entire upper extremity was packed in chipped ice and enclosed in a rubber pillow case. The patient on admission complained of rather severe pain in the right hand and wrist and this was greatly relieved following the packing of the right upper extremity in the ice chips. The patient remained fairly comfortable during the first 14-hour period in which cryotherapy was continued. During this period and for approximately the first 24 hours there was a rather marked amount of bloody serous drainage from the fang penetrations. During the first 14-hour period, there was a very slight swelling of thumb and hand only, without discoloration. After the first 14 hours an alternating regimen of ice packs for an hour, and no ice for an hour, was started, but was discontinued after a few hours because of severe pain reaction induced by the change of temperature and increased venom action. Cryotherapy was now discontinued.

[5] Approximately one hour after the bite, 20.4 mg. of dry venom were taken from the snake. Fourteen days later a second extraction yielded 87.8 mg. of dry venom. Thus, the victim was possibly subjected to 67.4 mg. in terms of dry venom.

Approximately 16 hours later the thumb became ecchymotic and several hemorrhagic blebs developed (Fig. 5) which in a few days progressed to a sloughing of the fat pad and skin, resulting in exposure of the flexor tendons (Fig. 6 and 7). During this period, some ecchymosis developed on the medial aspect of the arm and the lateral chest wall.

During the first 48 hours after the bite the patient's general condition was excellent. On the third day, he was lethargic; extensive swelling of the entire upper right extremity and right lateral chest wall occurred, but with no apparent impairment of blood supply to the right hand or nerve involvement.

FIG. 5. Ecchymosis and hemorrhagic blebs developed after approximately 16 hours following cessation of cryotherapy. This was the extent of bleb formation. Compare with bleb formation of Figure 1.

FIG. 6. A few days after bleb formation was followed by sloughing of the skin.

Blood chemistry tests and other additional criteria indicated a definite extracellular fluid deficit which was corrected by administration of intravenous glucose and saline. Prompt improvement followed.

By the second week a debridement was performed and a split-thickness skin

FIG. 7. Sloughing of the fat pad and skin resulting in exposure of the flexor tendons

FIG. 8. A functional thumb was obtained with no damage to the upper extremity.

graft was done which was later replaced by a pedicle transfer from the anterior abdominal wall. There was some general wasting of the entire right thumb with a loss of complete flexion; however, a functional thumb was obtained and no other damage to the upper extremity resulted (Fig. 8). Total hospitalization was 5 weeks.

Although the results of the two cases just described might be attributable to chance, they afford almost as good a controlled experiment as it is possible to obtain in clinical cases and it is interesting to note that Andrews and Pollard (1953) report the following observations regarding two other bites: "In two serious cases resulting from eastern diamondback rattlesnake bites the site of venom injection into two patients was approximately the same. In each case, the area of the bite was quickly excised, and each patient received antivenin. Refrigeration was employed in one case, and no destruction of tissue resulted. In the other case, without refrigeration, considerable damage resulted around the excised area."

TREATMENT

Tissue destruction and death resulting from snake bite involves two actions: the chemical action of the venom (Porges, 1953) and the action of bacteria. Rapid absorption of the neurotoxic factor may prove fatal. The action of the other venom components may produce conditions that later will result in death and/or mild to serious destruction of tissue which in turn may set the stage for further tissue destruction or infection. The technique outlined below is designed to control or prevent these conditions.

1. Place a ligature at once between the site of the bite and the body, but as near the point of entrance of the venom as possible. This is the quickest way to prevent rapid absorption. The usual type of tourniquet is not adequate and will permit the spread of the venom. A hemostat should be used, especially if the bite is on one of the digits, but obviously not to the point of traumatization (Raynolds, 1952).

2. Place a piece of ice on the site while preparing a suitable vessel of crushed ice and water.

3. Place the member well above the point of ligation in the iced water. Iced water initially is preferred to ice packs, since the thermal conduction in iced water is much greater and the rapid reduction of the tissue temperature is imperative.

4. After the envenomed member has been in the iced water for not less than five minutes, remove the ligature but keep the member in the iced water for at east two hours.

5. Pack the envenomed member in finely crushed ice. Since pit-viper venoms may stay in the area of the bite under normal body temperature for 14 hours or more (Jackson, 1929), the cryotherapy will have to continue for a minimum of 24 hours. The bite of a snake having a length of four feet or more may require three or more days of continuous cryotherapy. Since snakes inject different amounts of venom under varying circumstances, the test of venom action should be made

after 24 hours. This simply consists of a brief warming up period to see whether or not the patient can detect the feeling of venom action. Caution should be observed to see that the patient does not confuse the pain associated with the first five minutes of the warm-up period with venom action. Do not discontinue cryotherapy too soon.

Cryotherapy properly used will prevent a too rapid absorption of the lethal neurotoxic factor and prevent tissue destruction by the enzymatic action of the venom. Anderson (1953) states, "the speed at which an enzyme reaction proceeds is greatly influenced by temperature. The optimum temperature for most of the body enzymes under ordinary conditions is around 37° to 45° C." Ice bags are not adequate. The ice must not be permitted to melt away from the body surface, otherwise an igloo effect is created and the tissue temperature will rise to an enzyme-active level. Likewise, if the bite is more proximal on the limb, the entire distal portion and proximad to well above the site of injection must be packed in ice.

If ice is not immediately available, other methods for reducing the temperature may be employed. The most effective media are the bottled gases under pressure, such as those in fire extinguishers (carbon tetrachloride or carbon dioxide). Frigiderm, ethyl chloride or freon are especially useful. An efficient technique has been developed as explained previously (Stahnke, 1955).

6. Keep the patient comfortably warm at all times. After the first twenty-four hours following the bite, removal of the enzymes from the site will be hastened if the patient is kept somewhat uncomfortably warm.

Hypothermia per se will not prevent ultimate tissue destruction by the venom. To appreciate the importance of step six and to make the best use of cryotherapy, it is well to recall the "hunting" phenomenon described by Lewis (1930), namely, the fluctuations in temperature which occur in the immersed parts. More recent findings (Burton and Edholm, 1955) show that when first immersed in iced water there is an immediate constriction which may be so intense as to occlude the local circulation completely, and that the fluctuations of skin temperature are accompanied by periods of vasodilatation. Likewise, the frequency of the waves of constriction increase with the length of immersion so that the longer the finger or hand is immersed the more frequent are the periods of constriction. Thus, the use of continuous hypothermia alone would maintain at the site of the injection a dangerous concentration of venom which could lead to serious results when cryotherapy was discontinued. Spealman (1945), however, found that the ambient temperature affects this condition and that the thermal state of the body as a whole affects the local vasodilatation. The cold vasodilatation is greatly increased when the subject is hot, and reduced but not abolished when the subject is cold. Thus, keeping the patient adequately warm will allow gradual venom absorption while the low temperature is preventing tissue destruction through enzymatic action at the place of greatest venom concentration.

The question of treating a patient suffering from thromboangitis obliterans for pit-viper venenation has been raised. Obviously the conditions extant under the T.C.S. method would unquestionably lead to amputation. From Spealmans'

(1945) findings and the great improvement experienced during the hot Arizona weather by those afflicted, it would appear that if the ambient temperature were suitably high, cryotherapy would have much to offer and would not be contraindicated.

7. The warm-up period after cryotherapy is important. This must be done gradually. It can best be accomplished by allowing the water in which the member is immersed to come to room temperature gradually. This is especially important if the patient did not receive cryotherapy promptly. Some tissue destruction has taken place and capillary fragility has increased. Thus, a rapid warm-up technique would tend to increase tissue destruction.

Supplementary therapy

a. *Incision and suction.* When properly performed, these can be very beneficial, especially if the snake was large. Incisions should not be made by a lay individual. Before any incision is made, the tissue should be refrigerated for about 30 minutes in order to prevent enzymatic action during surgery. Multiple incisions should not be made, as the doubtful benefit derived is greatly offset by the danger of severance of nerves which might otherwise survive. Since venoms spread rapidly in tissues, the complicated fascial spaces of the hand and feet are already affected before incisions can be made. Unless placed by a competent specialist such incisions are liable to produce deformity. It has been observed that nerves may remain viable through the most heavily necrosed areas following snake bite (Tenery, 1955). The avoidance of nerves, therefore, is a valid consideration when incising extremities. Also keep in mind that since the fang is curved, the incision should begin with the fang puncture and move in a direction that would be posteriad on the longitudinal axis of the snake's head.

b. *Injection of local anesthetic agents and other fluids.* The treatment of bites on the hand or foot must take into account certain anatomical factors peculiar to these regions. Each digit, while endowed with a vigorous blood supply, is precariously dependent on the patency of its proper volar digital arteries and the venous drainage system. The rigidity of the skin envelope of the finger will bring about venous obstruction more readily in the finger than elsewhere. The same considerations are also applicable to the palm of the hand where the tough volar skin and the septa of the palmar fascia create unyielding closed spaces. It is a well established principle, therefore, that local anesthetic agents be used cautiously in the hand. Vasoconstrictors are never used and the amount of the agent injected is kept quite small to avoid the possibility of elevating the pressure in the closed spaces. By the same token it is unwise to inject antivenin into a hand or foot already tensely edematous. Even in freshly bitten areas, injection of antivenin or saline solution may actually hasten local tissue necrosis instead of preventing it as is commonly believed (Githens, 1941; Stahnke, 1955). The diminished capacity of edematous tissues to take up subcutaneously injected substances is well known and constitutes an additional contra-indication to local use of antivenin in bites of the extremities.

c. *Extracellular fluid deficits.* In case of pronounced edema, it is important to

keep up the fluid balance. Extracellular fluid deficit can in itself lead to a state of shock and even death. If such a deficit does occur it can be readily managed by Moyer's solution orally or by the parenteral administration of 5% dextrose solution.[6] In cases of heavy, pit-viper venenation where cryotherapy is not employed, whole blood is indicated because of the severe drop in the hematocrit reading brought on by erythrocyte destruction and increased capillary permeability.

d. *Analgesic agents and other extra therapeutic agents.* Snake venoms are complex compounds. Consequently, the principle of chemical compatibility should be observed to the extent that therapeutic agents not specifically tested for use with the particular venom under consideration should be used with extreme caution. Morphine or Demerol® should not be used during the first twenty-four hours. A possibility of synergistic action with the neurotoxic factor exists (Stahnke, 1955, 1956a.). ACTH and Cortisone in laboratory tests do not show antitoxic value (Stahnke, 1955; Schöttler, 1954). Schöttler also gives the warning that the use of antihistamine is strongly contra-indicated.

DISCUSSION

Since snake venoms produce their harmful local effects through the action of enzymes or enzyme-like substances, the use of cryotherapy is definitely indicated. Conversely, any technique that permits the venom enzymes to remain concentrated in the body at normal temperatures is providing optimum conditions for tissue destruction. Although incision and suction may remove some venom, yet the most efficient technician can hope to remove only a relatively small per cent of that injected. Cryotherapy conducted properly is able to cope with the situation. Additional advantages of cryotherapy are that it prevents rapid absorption of the lethal neurotoxic factor, inhibits bacterial activity, provides ample time for the correct administration of antitoxin, and benefits the emotional condition of the patient by providing a very effective anesthetic action. The usual severe pain experienced in pit-viper venenation is absent (Stahnke, 1955).

The use of cryotherapy for the control of venom is not new. It has been used throughout Arizona since 1936. In fact, if anyone is stung or bitten by some creature, it has become a fairly well established household practice to use cryotherapy immediately and ask questions later. When dealing with venenation, this, of course, is the intelligent thing to do. Even if the venom per se is not lethal, there is always the possibility of anaphylaxis. As a household and medical practice Arizonans have successfully used cryotherapy for the bites and stings of many kinds of venomous arthropods as well as venomous reptiles. The experience has been sufficient to show that cryotherapy when properly applied is effective and far superior to any single therapeutic agent at present employed. Some early experiments on refrigeration for snake bite did not make ideal use of

[6] Lactate Ringer's solution or normal saline may be used but 5% dextrose would be more desirable since in stress states the body hangs on to sodium rather tenaciously and if one overloads the extracellular compartment with salt a state of extracellular dehydration occurs.

either cold or tourniquet and adequate allowance was not made for animal size (Allen, 1939).

It is amazing that there is still so much misinformation on the subject of hypothermia, disseminated by persons who lack either personal experience or knowledge of the literature. Allegations of harm are mostly muddled impressions from "immersion foot", the result of long immersion of limbs in cold (often not icy) water, in association with dependent and confined position, relative immobility and usually depressed general physical and mental vitality. The facts have been reviewed in many publications, (Allen, 1945, 1946; Crossman, 1956) but some details of interest in snake bite treatment are as follows: (a) limbs with tourniquet-block of circulation have been kept at ice temperature for periods up to 54 hours, with ensuing recovery (Allen, 1938); (b) human limbs after iliac or femoral arterial occlusion have been kept at ice temperature for 14 to 28 days, and amputations (which would have been at upper-thigh level if done immediately) were performed above the knee, with healing (Mock and Tannehill, 1944; Ottaway and Foote, 1944); (c) a typical absurdity was shown in the criticism (Allen, 1945) of authors who kept dogs' legs in ice water (with confinement and dependent position) for seventy-two hours. The production of "immersion foot" under such conditions was nothing new, but these authors drew the remarkable conclusion that refrigerated limbs (even for the few hours required for surgical anesthesia) must be amputated, disregarding the fact that not one of their dogs' legs even under the artificially injurious conditions had required amputation; (d) there has been disregard of the facts that shipwrecked sailors in tropical seas also suffer from "immersion foot", and in experiments fully as great destruction is caused by long immersion in comfortably warm water as in cold water (Safford, 1950); (e) a leg supposedly requiring amputation for a burn was saved by eleven weeks of hypothermia, beginning with several days at ice temperature and continuing at milder degrees (Kross, 1945; Crossman and Allen, 1945). These and numerous other experiences should remove any apprehension concerning such uses of refrigeration as may be required for snake bite and make untenable any suggestion that cryotherapy would enhance the destructiveness of snake venom.

The ligature is an exceedingly valuable adjunct since it provides the quickest means of localizing the venom until the temperature of the site can be adequately lowered. The delay of absorption by a "venous" or loose tourniquet is imaginary or unimportant. With the use of cryotherapy extensive tissue destruction from the use of a ligature can be largely eliminated. In addition to this, if a shoelace or cord is used, the simple expedient of using a cloth or paper shield between the ligature and body surface will prevent trauma to surface tissue. Since hypothermia makes a long application of a ligature unnecessary, the possibility of nerve damage is precluded regardless of the type of material used.

SUMMARY

Tissue destruction, not death, is stressed as the major medical problem arising from snake bite in the United States. Case histories are presented contrasting

the effects of pit-viper bite when treated with or without cryotherapy. A seven-step regimen to prevent death and tissue destruction through the use of ligature and cryotherapy is presented. Emphasis is placed on the importance of using iced water initially, followed by a complete and continuous coverage of the envenomed member with finely divided ice. It is also stressed that success in the use of cryotherapy is dependent upon a careful consideration of the ambient temperature. Supporting therapy is also suggested stressing the following: (a) a more effective and conservative use of incision and suction; (b) the possibilities of increased tissue destruction by the improper use of antivenin; (c) a warning against utter disregard of the principle of chemical compatibility when selecting supporting therapeutic agents. A documented discussion of the wide margin of safety involved when using cryotherapy and the value of the ligature are given in conclusion.

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DOUBLE INFESTATION OF A VARICOSE ULCER WITH SCREW-WORM AND HOUSE FLY MAGGOTS

ALFRED H. LAWTON, MORRIS W. DEXTER AND LYMAN O. WARREN[1]

A considerable literature describing myiasis due to *Callitroga americana* in Florida has arisen since its first introduction from Texas during 1933 (Swanson and Goen, 1952; Taylor, 1938, 1950; Turnley, 1954). The reporting of infestation with common fly maggots of dirty wounds was observed so frequently in a bygone era that in historical medical literature the use of maggots to debride infected wounds was once considered an accepted clinical practice. However, the combined, and in some ways supplementary, accidental occurrence of these two forms of myiasis in a common ulcer site appears to be unusual enough in itself to be worthy of consideration. Likewise it is worthwhile to keep reminding ourselves that such filth infestations can occur even with our high level of hygienic attainment.

CASE REPORT

The patient had been seen on two previous occasions with varicose ulcers of the legs which had healed completely during each hospital stay. On the occasion reported, he presented himself with a large dirty recurrent varicose ulcer of the right leg. This ulcer was deep and contained necrotic tissue in which were numerous maggots. The story was that the ulcer had appeared only a few days prior to his admission. The patient had attempted self-care including a constant dressing, and had continued working on the highway crew as a bulldozer operator. The stasis ulcer increased in size and began to be quite moist. On the advice of a well meaning friend the patient had stopped dressing the wound so that the ulcer would be "exposed to the air" but had continued his dusty labor. About three days prior to this admission he had noted during lunch that several flies were quite persistent in their attention to his ulcer. Thereafter the ulcer developed rapidly in size and the material discharged increased so that the patient presented himself for care denying that he knew of the presence of the maggots in the lesion.

Physical examination was essentially normal except for the notation that the patient was a grossly obese (over 300 lbs.) filthy white male with a deep, dirty, necrotic lesion on the right leg approximately four inches in diameter and surrounded by a large brawny area of brownish sclerotic pigmented skin. Maggots were noted swarming in the necrotic material and when this was cleansed away, several burrows were observed penetrating into the normal underlying subcutaneous and muscular tissue. From each of these, several maggots of an entirely different appearance were removed. Laboratory and X-ray findings were considered to be normal.

Treatment in the hospital consisted of debriding the ulcer with individual

[1] From Veterans Administration Center, Bay Pines, Florida.

removal of the maggots on several occasions. Flooding the ulcer with ether caused the maggots to be loosened from the sinus tracts. When the last worm had been removed the ulcer healed rapidly on treatment which consisted of bedrest, whirlpool baths to legs, cleanly care, and a weight reduction program. It is worthy of note that with the weight loss the patient regained considerable self respect so that it is doubtful that he will neglect himself so grossly again.

DISCUSSION

The maggots removed from the patient's ulcer were identified as those of the common house fly, *Musca domestica*, and those of the screw-worm, *Callitroga americana*. The former larvae were cylindrical, smooth, tapered anteriorly and had no spines whereas the latter had the spines which give the typical screw-like appearance and the bulbous anterior end so that even in the ulcer it was obvious that a double infestation of fly maggots existed.

Examination of the ulcer revealed that the smooth larvae were feeding and living in the necrotic material of the lesion while the screw-worm larvae were all in the burrows which penetrated directly into normal appearing subcutaneous tissue, fat, or muscular tissue.

Questioning of the patient failed to ellicit any accurate statement as to the appearance of the maggots in the ulcer. The only evidence is his rather vague statement that he quit wearing a dressing over the necrotic lesion a "few days" before he came to the hospital and that "green flies swarmed" about his leg during his lunch period three or four days before his admission.

The patient operated a bulldozer working on secondary roads. Since this was a dry season, there were great clouds of dust and he had no opportunity for cleanliness during the day. Lunch was eaten along the roadside while he reclined against a tree. Farm houses and the usual farm animals were always nearby.

It is of considerable importance that one of the maggot types often feeds on necrotic tissue and the other penetrates into normal tissue and produces necrotic material. Therefore the two could live in happy symbiosis never completely cleaning up the necrotic lesion as the musca larvae alone might well have done for that was once their surgical usage.

The patient describes the flies as green or greenish blue. No further identification could be obtained. While only one adult of the larvae described fits the color description, both are common in Florida, and both are attracted to necrotic animal tissue for egg laying so the double myiasis observed is easily possible in a person working outdoors and paying little or no attention to cleanliness of his person.

SUMMARY

The individual whose case is summarized presented himself with a necrotic varicose ulcer of the lateral aspect of the right leg in which maggots of *Callitroga americana* and of *Musca domestica* were living in symbosis. The *Callitroga* maggots had penetrated deeply into healthy muscle and fibrous tissue and were

feeding avidly on normal tissue. The muscoid maggots were present superficially feeding in the necrotic material much of which was being provided by the activity of the *Callitroga* maggots.

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PRESENTATION OF THE WALTER REED MEDAL FOR 1956 TO DR. KARL F. MEYER[1]

N. PAUL HUDSON

Chairman, Walter Reed Medal Committee

Mr. President and Members of the American Society of Tropical Medicine and Hygiene:

The Walter Reed Medal Committee, composed of Dr. Sodeman, Dr. Taliaferro and myself, was pleased to nominate Dr. Karl F. Meyer for the 1956 award. The nomination was unanimously approved by the Council of the Society, and I as Chairman have the honor to present the medal to Dr. Meyer.

The Walter Reed Medal award was established by the American Society of Tropical Medicine and the first medals were conferred in 1936. This year we honor Dr. Meyer in compliance with the principal condition of award, namely, "in recognition of meritorious achievement in tropical medicine".

Karl F. Meyer was born in Basel, Switzerland, in 1884. He received the degrees of Doctor of Veterinary Medicine and Doctor of Philosophy from the University of Zurich in 1909 and 1924, respectively. Several honorary degrees, including Doctor of Medicine, have been conferred on him by institutions of higher learning in this country and abroad in acknowledgment of his widely known contributions to biological science and public health.

After serving as government pathologist in the Union of South Africa from 1908 to 1910, Dr. Meyer was on the faculty of the School of Veterinary Medicine of the University of Pennsylvania for three years. Thence he moved to the University of California where he has held numerous academic and administrative appointments in bacteriology, tropical medicine and medical research, including his term from 1924 to 1954 as Director of the George Williams Hooper Foundation for Medical Research. He now holds the titles of Director Emeritus of the Hooper Foundation and Professor Emeritus of Experimental Pathology.

Dr. Meyer's career has been marked by a steady and intense program of biological research, recorded in scores of original scientific papers and review articles from his first in 1908 to the present time. His investigations have been concerned with problems in the fields of bacteriology and immunology, protozoology, mycology, virology, and comparative pathology. Under his name alone or with those of his colleagues, research on animal infectious diseases, brucellosis, typhoid and intestinal bacteriology, food poisoning, and fungous diseases has been reported. Scientific circles have accorded special consideration to the valuable achievements of his laboratory in the study of botulism, psittacosis and ornithosis, epidemic and sylvatic plague, and in the isolation of the virus of Western equine encephalomyelitis. Dominant in his research and its inter-

[1] The Walter Reed Medal was presented to Dr. Meyer at the dinner meeting of the American Society of Tropical Medicine and Hygiene held in New Orleans, Louisiana, on November 2, 1956.

pretation have been the elucidation of animal diseases as sources of infections in man and the application of this information to the prevention and control of epidemic human disease.

The contributions of Dr. Meyer have not been limited to his laboratory findings and scientific papers. Education has been an important part of his career, both in formal teaching and in informal associations. He has extended his influence also by applying laboratory science to public health and has successfully pressed for the adoption of scientifically sound principles and practices in community hygiene and industrial sanitation. The numerous city, state, national and international committees and commissions on medical research and public health of which he has been a member or consultant have always benefited by his wise counsel.

Dr. Meyer holds membership in many national, foreign and international scientific societies, frequently with the added distinction of being elected Fellow. He has long been a member of the American Society of Tropical Medicine and Hygiene and was a charter member of the American Academy of Tropical Medicine. Several national associations have elected him their President, and honor societies of this country and abroad have extended membership to him.

The American Society of Tropical Medicine and Hygiene takes pleasure in presenting the Walter Reed Medal of 1956 to Dr. Karl F. Meyer in acknowledgment of his contributions, made in a long scientific and public career, to comparative pathology and epidemiology and to scientific measures for the promotion of the public health; and especially in recognition of his contributions to tropical medicine and hygiene in his original work on psittacosis, plague and equine encephalomyelitis, and his leadership in international science and control of world-wide disease.

ACCEPTANCE OF THE WALTER REED MEDAL[1]

DR. KARL F. MEYER

I am moved by your generous and kind words and must at once express my deep appreciation of the great honor the Society has done me in awarding me the Walter Reed Medal of 1956. I am filled with humility when I think of the former recipients: Mrs. Walter Reed, the Rockefeller Foundation, William Bosworth Castle, Herbert Charles Clark, Carlos J. Finlay, Gustavo Capanema, James S. Simmons, Paul F. Russell, N. Swellengrebel, Rolla E. Dyer and Lewis W. Hackett—names too well known throughout the world in the field of tropical medicine and public health to need comment here.

Tropical medicine is now in a phenomenally precocious infancy. Its future is charged with excitement and challenge, and on its shoulders rest many of the responsibilities for the development of the major geographic areas of the earth. It is faced with some of the most prevalent infectious diseases that afflict man and his animals. The future of the tropics depends to a large extent on knowledge of its diseases and on the application of this knowledge. Imagination is taxed in visualizing the volume of work yet to be done in this field. The growing uniformity of some present-day societies and the desire for comfort and security are perhaps not the most favorable conditions for cultivating the necessary adventurers. The work will have to be done, as it has always been, by people with enough spirit to live vigorous, but often lonely, lives without the familiar comforts, by people who derive their satisfactions from facing difficulties, taking risks even with their lives and to whom work is life.

Every human being has goals he strives to achieve to give meaning to his life as an individual. The guidance, friendship and instructed support of other scientists through these many years, first in Europe and then in America, have contributed to the achievement of some of these personal goals. In this group are the individuals and groups who led the way, who have given inspiration, counsel and support, who have stimulated thought and action and who have worked and worked and worked. There are those unique personalities, integrated individualists and original thinkers who provided the basic structure of knowledge and principle. I would like to take this opportunity to refresh your memories of some members of this large group honored today by the Walter Reed Medal.

A student with an interest in the natural sciences, living in the heart of Europe in a country without colonies, first learned something about the problems in tropical medicine through evening conversations in remote Alpine cabins with British mountaineers on leave from their assignments in the Zonae torridae. Courses in general zoology, in particular lectures and demonstrations in the new discipline of protozoology under Professor Friedrich Zschokke, had a special fascination because they vividly described the impact of malaria on the people living in the warm climates. Unforgettable remains the opportunity to study

[1] Delivered at the dinner meeting of the American Society of Tropical Medicine and Hygiene held in New Orleans, Louisiana on November 2, 1956.

the illustrated monograph by Battista Grassi showing the complete life cycle of the human malarial parasite in *Anopheles* and the chance to see some of his original preparations during a holiday visit to Rome.

During a conversation with Dr. George Henry Falkner Nuttall, Quick Professor of Biology at the University of Cambridge, he suggested that I read the first paper of a series on fundamental researches in the biology, structure and classification of flies, fleas, lice and ticks as vectors of disease. The paper he handed me carried the title "Relation of Insects to Plague". Whether the reading of the paper or the stimulation of meetings with Doctor Nuttall was responsible for the imprint of these ideas is not too clear as I think back now to 1906. Professor Nuttall planted ideas in my mind that were decisive as a basis for understanding many tropical diseases. He made it clear that topography, climate, vegetation and other environmental factors determine whether an area will be likely to sustain the malarial parasite. When I was learning what was known about the factors that cause trypanosomiasis to flourish—about the tsetse fly, trees, shade, proximity to water and cattle and wild game—Professor Nuttall intimated that animals might well serve as reservoir hosts of the parasite. He foresaw many of the relationships between animal and man bridged by an insect vector. That this interplay was profoundly involved in the development and public health of Africa was amply emphasized. The implications of conversations with this able scientist haunted me during the next fifty years. They haunt me still.

More zoology, protozoology and entomology were crowded into the regular curriculum. Under the guidance of Professor H. Zangger at Zurich, microchemical techniques of staining protozoa were tried and mastered. As early as 1904 a silver staining method had proven useful in revealing bacteria in cells; it became later the well-known Levaditi procedure for revealing spirochetes in nerve tissues.

Formal training came to an end, and the decision was made to prepare for an academic post. An appointment in the well-known Institute for Infectious Diseases at Berne as assistant to Professor Wilhelm Kolle was chosen because it offered the best opportunity to serve under the guidance of a scientist who had seen the problems of the tropics. Professor Kolle, while serving Robert Koch in Africa as associate in the development of an effective method of reducing the ravages of rinderpest by active immunization, had already realized the unlimited variety of protozoal infections in this region. He vividly described many tropical diseases, at that time of unknown etiology, in retrospect probably due to viruses.

My desire to become personally acquainted with these mysteries was promptly fulfilled when Professor Kolle graciously recommended me to a post as pathologist at the institute of the Transvaal Department of Agriculture at Onderstepoort. There I saw Professor Kolle's predictions come true, one after another. Opportunities to learn and to see new things were unlimited. A diagnostic and vaccine production service for the area later to become known as the Union of South Africa, brought in hundreds of specimens, each representing to the novice a research problem in pathology. What may now appear commonplace, for example the isolation of *Brucella melitensis* from the blood of a patient, was a

discovery in 1909. Epidemiologic inquiries carried out in connection with maintenance of a Pasteur antirabies treatment center brought to light that the rabies virus existed in many wild animals and could be transmitted to man independent of domestic dogs and cats.

The daily experiments by the entomologists at the Institute dispelled any vagueness in the new concept that arthropods are vitally concerned in transmitting disease. The significance of the arthropod in infectious diseases was just coming to light. Certain ticks readily acquired the ability to transmit the small piroplasms present in myriads in the erythrocytes of cattle suffering from African East Coast fever. And from this disease it was clearly learned that even in infections by similar organisms one must anticipate differences. Different from Texas fever and other piroplasm infections, in East Coast fever the peripheral blood of infected cattle transferred to susceptible cattle by every conceivable route in quantities of up to 1 liter failed to cause infection. But surgical implantation of pieces of spleen or lymph nodes, even injection of emulsions of these tissues, induced typical East Coast fever after an incubation typical of this infection. A study of the life cycle of the protozoan disclosed that asexual and sexual multiplication, manifestations of which were originally seen by Koch and described as Koch's granules, takes place in the cytoplasm of the lymphocytes and endothelial cells. This stage terminates in the formation of gametocytes, and it is these that invade the erythrocytes. In its erythrocytic phase in the mammalian host the parasite cannot multiply further. In time it degenerates in the blood and organs or even when transferred to another susceptible bovine host. However, when, through a blood meal, the parasite is taken into the intestinal tract of *Rhipicephalus appendiculatus* or other ticks capable of transmitting the parasite, the gametocyte transforms into zygote, ookinete and finally into sporoblasts. The sporoblast, when introduced into a new host with the saliva of the tick, initiates infection.

As a background to this investigation it became imperative to study the monograph and papers on Texas fever by Theobald Smith, who, in the judgment of his European equals, stood foremost in the list of American biologists. In Europe this contemporary of many of the founders of microbiology was considered the greatest American bacteriologist of his time. His claim to immortality as a scientist was his demonstration that blood-sucking arthropods transmit protozoal disease. In 1889 Theobald Smith described a protozoan now known as *Babesia bigemina*, discovered in the blood of cattle suffering from Texas or redwater fever. This in those days was remarkable enough to secure his position. But, having heard the unlikely story that the disease was carried by a tick that lived on and sucked blood from cattle, he set to work with F. L. Kilborne on an unsolved problem that appeared to be in some way connected with the soil. In long series of laborious investigations not only did he prove that Texas fever was in fact conveyed by ticks, but he also pursued the idea further to the conclusion that female ticks having sucked blood of a diseased cow fall to the ground, there deposit thousands of eggs and, on their development some 30 days later, if they are able to migrate to healthy cattle, pass on Texas fever to their host.

For months the bulletin first issued in 1893 by the U. S. Department of

Agriculture was my daily companion, not only because it furnished in simple form an answer to an enigma in nature, but also because it served as a model of scientific reports. Reading this document and Theobald Smith's later studies on immunization against numerous diseases, one felt the power of one of Nature's high priests, always devoted to simple understanding and constructive thinking. Complexities and uncertainties were realities to him, but the direction of his thought was always toward finding some means of reducing them to elementary simplicity.

No scientific papers had a greater impact on the planning of my future than those of Theobald Smith. The forthcoming opportunity to come to the United States and to meet this great scientist was eagerly accepted. From 1910 until his untimely death in 1934, at first in frequent visits to Harvard to discuss undulant fever and later in his home at Princeton, every conceivable field of pathology and immunology was covered. I was proud and grateful to be one of a small group of his confidants. Through these informal meetings I became familiar with his personal views of biology of disease, particularly of the host-parasite relationship, many years before he summarized them in the Vanaxem Lectures as "Parasitism and Disease". Powerful as a guidepost for life was his philosophy expressed in these words:

> To those who have the urge to do research and who are prepared to give up most things in life eagerly pursued by the man in the street, discovery comes as an adventure rather than as a result of a logical process of thought. Sharp, prolonged thinking is necessary that we may keep on the chosen road, but it does not necessarily lead to discovery. The investigator must be ready and on the spot when light comes from whatever direction.

Now to go back a little, on my way from Africa to the United States I spent a few weeks visiting the Institute for Experimental Therapy in Frankfurt am Main to report to Professor Paul Ehrlich on some studies with 606 that he had sent to South Africa. Here some more important impressions were imprinted on my memory. My travel notes record that every staff member there had been taught accuracy and precision as essential in scientific work. Only repeated testing and hard work prevent guess work and self deception. There is no need for preliminary reports. On the other hand, it is impossible to stay in a field until the entire crop is brought in. One must always leave some part of the harvest for others. These sound principles are sometimes forgotten these days.

After a spirited discussion of protozoal infections at a meeting of the American Association of Pathologists and Bacteriologists in 1911, Professor Novy invited me to spend Easter Sunday and Monday in Ann Arbor. It was doubtless his account of his experiences with plague, both in San Francisco and later in his laboratory, that kindled my interest in this infection. These early impressions were broadened and the basic knowledge matured through the guidance of Dr. George McCoy, then stationed in the U. S. Public Health Service Laboratory in California. Engaged in methodically unraveling the complex interplay between wild rodents and commensal rats, he freely made available the facts known at that time. Methods of discovering the existence of occult plague had not been

developed, and the early attempts to understand explosive local epizootics remained shrouded in mystery until Charles Elton at the University Museum at Oxford outlined the ecologic approach. Many years later, in fact after two epidemics of pneumonic plague had created more important problems, his teachings were applied in cooperation with a group of enthusiastic entomologists and mammalogists. The rapidity with which the strides were taken from those beginnings speaks of the quality of the participants. In only a little over fifty years many of the mysteries were dispelled. The foci of plague are characterized by definite ecologic peculiarities in fauna, topography, climate and vegetation. In fact these peculiarities are now so well characterized that when the assembly is recognized it can now be predicted that if plague does not already exist there, it could find ideal support for its survival if it were to be introduced. Although the tropics have provided this assembly, they share this unwanted distinction widely, as mortality figures in the history of Europe tragically show. Although many means are now known to curtail the intercontinental travel of the plague bacillus, it continues in its ancient rural environment where it is entrenched beyond our reach for the time being. Now we are having to learn discretion in applying the measure that is at last saving lives that even two decades ago would have been lost to plague. If antibiotic therapy is employed too energetically, the deadly toxin of the organisms is released in great quantity and is as damaging as ever.

While I was on sabbatical leave in 1923 my interest in the widespread inquiries by European microbiologists into the cause of postvaccinal encephalitis and von Economo's encephalitis brought me into contact with Dr. J. C. G. Ledingham at the Lister Institute in London and with Professor Robert Doerr at the Hygiene Institute in Basel. Both introduced me to the new method they were using to search for invisible infective agents causing lesions in the central nervous system. The direct subdural or intracerebral injection of small laboratory animals with encephalitic material was a new procedure. Unfortunately much of the early work on the transmission of infection was confused by a concurrent meningoencephalitis in rabbits and had to be disregarded. So emphatic was the warning about inapparent infections of the central nervous system that a few years later when it became urgent to establish the viral nature of Western equine encephalomyelitis the pitfalls of the period of 1917 to 1922 were still fresh in mind and could be avoided. Using a variety of large and small animals, particularly guinea pigs and mice, the virus was isolated without difficulty. This was the first isolation of an agent belonging to the lengthening list of arthropod-borne neurotropic viruses. Without the guidance given by the early masters of virology, successful isolations would have been delayed.

One other, among a host who must remain unnamed in this short reminiscence, must not be forgotten. Ever since I met him shortly after my arrival in this country he has served me as a model. Dr. Hans Zinsser at Stanford, Columbia and Harvard, a brilliant teacher and courageous investigator, was a man of extraordinary mind, independent, outspoken, tolerant, and a judicious author. He exemplifies in his lovable way the truly convinced internationalist. The breadth of his interests is reflected in "As I Remember Him", and in the ac-

tivities of his students who are to be found as leaders in experimental laboratories, working in field epidemiology or engaged in clinical practice everywhere. Impressed by the wisdom of his words, I wrote some of them down, and I have read them over and over again. One of these comments, made in 1935 at the Second International Congress for Microbiology in London, comes to mind on this occasion:

> And may some political Leeuwenhoek discover a microscope by which he can see and define the little animalculae of enmity and hatred and savagery, so that at last, in International Congress assembled, wise men may sit in peaceful assembly planning research into the virulence of human stupidity and consider measures of active immunization, chlorination and delousing for international politics as we do this for infectious disease.

BOOKS RECEIVED

Annual Review of Entomology, ed. by Edward A. Steinhaus and Ray F. Smith. 407 pages. Palo Alto, Calif., Annual Reviews, Inc., 1956. $7.00.

Soybeans for Health, Longevity, and Economy, by Philip S. Chen and Helen D. Chen. 241 pages illustrated. South Lancaster, Mass., The Chemical Elements, 1956. $3.00.

Virus die Geschichte vom Geborgten Leben, by Wolfhard Weidel. 186 pages illustrated. Berlin, Springer-Verlag, 1957. DM 7.80.

Venoms, ed. by Eleanor E. Buckley and Nandor Porges. 480 pages illustrated. Washington, D. C., The American Association for the Advancement of Science, 1956. $9.50, $8.25 to AAAS members.

Practical Dermatology, by Samuel M. Peck and Laurence L. Palitz. 380 pages illustrated. New York, McGraw-Hill Book Company, 1957. $7.00.

BOOK REVIEWS

Diseases of the Skin, by RICHARD L. SUTTON, JR., A.M., M.D., F.R.S. (Edin.), Chairman of the Department of Dermatology, University of Kansas Medical Center. 11th edition. 1479 pp., illustrated. St. Louis, C. V. Mosby Company, 1956. $29.50.

Sutton's remarkably lengthy and complete textbook of dermatology boasts 1479 pages, much of it in small type, and 1972 illustrations. In his preface, the author describes how a volume such as this came into being. For years, Sutton has abstracted every article related to skin disease that he has seen. His description of a disease often consists of a reference to the original report of the condition, a statement of its ordinary manifestations, and several references to the variations the process may exhibit. The result is a textbook in which almost everything pertaining to dermatology is at least mentioned. A vast array of references to the literature is included within the text material in parentheses instead of in footnotes or at the end of the chapter. This method of writing is at once the book's greatest virtue and its most serious weakness. Valuable indeed is the host of references so conveniently placed and the inclusion of so many subjects, but at the same time it makes for a somewhat less orderly, reasoned, and didactic approach to the description of disease entities.

The book is remarkably up-to-date and makes effective and extensive use of illustrations. The photographs, although black-and-white, are wonderfully clear and well chosen and do much to amplify the text. Especially noteworthy are the beautiful photomicrographs illustrating the excellent chapter on the anatomy of the skin.

Dr. Charles R. Rein wrote the interesting and comprehensive section on the serodiagnosis of syphilis. He includes a good discussion of the problem of false positive reactions and describes the use of specific tests for syphilis such as the TPI test.

GUSTAVE SIROT

Medical Research: A Midcentury Survey. Published for the American Foundation of 565 Fifth Avenue, New York City 17, and Westbrook, Connecticut, by Little Brown and Company, Boston and Toronto. 2 Volumes: Vol. 1—American Medical Research: in principle and practice pages 765; Vol. 2—Unsolved Clinical Problems: in biological perspective pages 740. Price $15.00.

In attempting to review so competent and comprehensive a survey by Esther Everett Lape and her associates Eleanor M. Phillips and Margaret Thomas Edgar, and the secretarial staff, Helen C. Hahn, Hazel Berry, and Evelyn R. Hahn, one thinks of Robert Louis

Stevenson's remark on matrimony, that "when a man marries he domesticates the Recording Angel." These 1504 pages convey a remarkable number of significant facts so well chosen and so skillfully presented as to excite awe as well as admiration.

Miss Lape's range and discrimination is apparent in the choice of the following names of the Committee of Consultants: Walter Bauer, George H. Bishop, Austin M. Brues, George W. Corner, Rene J. Dubos, C. A. Elvehjem, Louis F. Fieser, Thomas Francis, Jr., Ralph W. Gerard, Michael Heidelberger, William G. Lennox, Esmond R. Long, William S. McCann, Thomas Parran, Linus C. Pauling, John P. Peters, I. S. Ravdin, Francis O. Schmitt, Truman G. Schnabel, W. M. Stanley, DeWitt Stetten, Jr., E. L. Tatum, Stafford L. Warren, Paul A. Weiss, M. C. Winternitz, John B. Youmans.

Equally notable is the appreciation by these Consultants of the survey in point of the content and treatment of the very fields in which they are most competent to criticize.

The ten subjects chosen for discussion in Volume 2 are: Current Metabolic Concepts Orienting Research in Biology and Medicine; Cancer; Infertility; Arteriosclerosis; Hypertension; The Rheumatic Syndromes; Tuberculosis; The Nature of Viruses and of Virus Diseases; Alcoholism; Biology of Schizophrenia.

To have written chapters whose content and form are warmly praised by experienced performers and competent critics in the field under discussion justifies one's admiration, but the form of presentation offers a singular further advantage: the volumes lose the usual formidable characteristic of any presentation of 1504 pages because they can readily and profitably be dipped into in shorter sessions. Because of the arrangement of the large number of subjects and aspects of medical research the book is not only possible but interesting and rewarding to read in several sittings.

By so much as you feel that as a specialist you haven't time to know much of the ecology of your job or the circumstances of men and work in other fields of medical research this mid-century survey offers you a very considerable consolation and enlightening correction.

I can't help hoping that by the year 2006 A.D. a volume of comparable scope and competence will appear to present the medical research of the remaining half century. It would be so interesting as a sequel.

ALAN GREGG

TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Fifth Annual Meeting

New Orleans, Louisiana, October 31–November 3, 1956

OFFICERS

President—Asa C. Chandler
President-Elect—Justin M. Andrews
Vice-President—Willard H. Wright
Secretary-Treasurer—John E. Larsh, Jr.
Editor—Lewis W. Hackett

COUNCILORS

C. G. Huff
A. B. Sabin
M. C. Balfour
P. C. Beaver
S. W. Simmons
J. J. Sapero
T. H. Weller
W. C. Reeves

FORMER PRESIDENTS OF THE SOCIETY

Martin D. Young 1952
William A. Sodeman 1952–1953
Frederick J. Brady 1953–1954
William H. Taliaferro 1954–1955

MINUTES OF THE ANNUAL COUNCIL MEETINGS

The annual business meetings of the Officers and Councilors were held at the Jung Hotel, and were presided over by the President, Asa C. Chandler. The first meeting was held Wednesday morning, October 31, and the final meeting was held that evening. Officers and Councilors present were: Justin M. Andrews, Paul C. Beaver, Lewis W. Hackett, Clay G. Huff, William C. Reeves, Albert B. Sabin, Samuel W. Simmons, Thomas H. Weller, and Willard H. Wright. Rolla B. Hill, the in-coming Secretary-Treasurer (to take office January 1, 1957) also was present by invitation. Past-Presidents in attendance were: Frederick J. Brady, George R. Callender, Herbert C. Clark, E. Harold Hinman, N. Paul Hudson, and Edward I. Salisbury.

The minutes of the fourth meeting held November 2–5, 1955 in Boston, Massachusetts were accepted as published in the March, 1956 issue of the Journal.

The Secretary's report was presented as follows:

This report covers the period from October 16, 1955 to October 15, 1956. Highlights of the year were: (1) new publishing agreement, (2) new membership list, and (3) arrangements for the annual meeting. Other items of interest are included by number (4–10) below.

(1) *New Publishing Agreement.*—The present 3-year publishing agreement with Williams and Wilkins Company expires December 31, 1956. It was necessary, therefore, to consider a new agreement for next year. On February 20, 1956, the Editorial Board voted unani-

mously in favor of renewing the present agreement for a 3-year period, and on March 8 the Council voted likewise. On April 10, a new contract was signed, a copy of which is on file in the office of our Editor.

(2) *New Membership List.*—During the Boston meeting, the Editorial Board voted to continue the policy of publishing this list biennially. The last list was prepared in September, 1954, hence it was necessary to compile a new list during this year. This was completed in August (with 1197 names) and sent to the Editor of the NEWS for publication in a supplement to be mailed this year with an issue of the NEWS. Necessary changes in the list are being made until the time of publication.

(3) *Annual Meeting.*—General plans for the New Orleans meeting were initiated in mid-January when the Program Committee agreed on certain events and scheduling (see report of this Committee below) The Council later approved the recommendations of the Committee and selected N. R. Stoll as Moderator for the Symposium, "Helminthic Infections as the Cause of Disability and Disease." The announcement of the meeting was sent to all members on March 27, 1956. From then until the opening of the meeting, the necessary arangements were made by correspondence with the Local Committee, and various other committees and individuals.

(4) *Commemorative Medals.*—By mid-year the remaining medals, commemorating the fiftieth anniversary of the founding of the ASTM, were sold. Thus, a total of 150 medals was sold (original order of 100, one re-order of 50) in the two and one-half year period following the anniversary celebration in Louisville, November, 1953.

(5) *Receipt of Materials for the ASTM Archives.*—As per agreement of the Council at Boston, certain archives of the ASTM were shipped to the Secretary-Treasurer by Dr. E. C. Faust. These were added to the archives of the Society.

(6) *Election of New Secretary-Treasurer.*—The present Officer was re-elected last year with the understanding that his resignation would be automatic after serving one year (1956) of a second term. To replace him, effective January 1, 1957, the Council elected Dr. Rolla B. Hill.

(7) *Fellowship Certificates.*—Following election of the first 101 FELLOWS, by a vote of the Council at the Boston meeting, the certificates, prepared by last year's Committee on Society Organization, were engrossed and mailed during February.

(8) *Membership.*—As of October 15, 1956, the Society had 1074 active members in good standing. Also, the rolls include 16 honorary members, 7 emeritus members, and 1 life member making a grand total of 1098 members in good standing. In addition, there are 119 active members, delinquent in dues for 1956 (62), or for 1955 and 1956 (35), or for 1954, 1955, and 1956 (22), who failed during the past year to respond to statements of dues sent on three occasions. Therefore, with 1098 members in good standing, and 119 members in arrears, we have a total membership of 1217. This compares favorably with the total membership for each year following the formation of the new Society.

Included in the above total of 1074 active members in good standing are 62 new members elected after publication of the 1955 Transactions. The new members are as follows:

ALIO, IVAN S.
ASH, LAWRENCE R.
BABIONE, R. W.
BERGER, FREDERIC WILLIAM
BOWERS, ROBERT CARL
BROWNE, HARRY GRAY
CABRERA, HUGO A.
CABRERA PALMA, NOE DE JESUS
COLEMAN, ROBERT MARSHALL
DRENNAN, LAWRENCE MICHAEL, JR.
FELDMUTH, GUILLERMO MIGUEL
GALLOWAY, REGINALD BRUCE
GOODCHILD, CHAUNCEY G.
GOULSON, HILTON THOMAS
GRIFFIN, HERSCHEL EMMETT
LOEBER, THOMAS STANTON
MCCLINTOCK, MARY JANE
MACIS, SALVADOR ALBANES
MARTINS, DEOLINDA DACOSTA
MATHIES, ALLEN WRAY, JR.
MINNICK, EDWARD ROBERT
MONTAGUE, JOSEPH FRANKLIN
MUGGIA, ALDO
NAUENBERG, WALTER G.

ORIHEL, THOMAS C.
OSIMANI, JUAN JOSE
PENA-CHAVARRIA, ANTONIO
PETERS, EDWIN FERRELL
PISTEY, WARREN ROBERT
REEVES, RICHARD E.
REINHART, HARRY LOUIS
HAGGERTY, M. B.
HARDING, HARRY B.
HARRY, HAROLD WILLIAM
HEINBACH, WILFRED F.
HERRING, LIVIOUS DUNCAN
HOOGSTRAAL, HARRY
HSIE, JEN-YAH
JACQUES-ALMONTE, FERMIN A.
JAMUNI, ABHAYA P.
JANSSENS, PETER GUSTAVUS
JELLIFFE, DERRICK BRIAN
JENSEN, THORKIL
KATZ, FRANK F.
LANMAN, BEN MARR
LINKFIELD, ROBERT LOOMIS
ROWAN, WILLIAM BOYD
RUSSELL, CATHERINE M.
SCHWABE, CALVIN WALTER
SELTSER, RAYMOND
SEYMOUR, DAVID MERRILL
SHAMI, KHALID ASA'D
SOEPARMO, H. T.
SUTTON, RUFUS R.
TAKOS, MICHAEL J.
TRAGER, WILLIAM
TULLOCH, GEORGE S.
WALTON, BRYCE C.
WILLIAMS, CHARLES L., JR.
WITHERINGTON, CHARLES GORDON
WONG, LOIS
YOUNG, WILLIAM WOOD

The Secretary announces with regret the loss by death of three active members:

KOMP, W. H. W. (FELLOW)
LE PRINCE, JOSEPH A. A. (FELLOW)
FRENCH, WILLIAM O.

The following submitted resignations:

BAKER, ROGER DENIO
BELL, FRANCES
BRACKETT, STERLING
CARR, HENRY P.
CLARK, SIDNEY B.
DUTCHESS, CHARLES E.
HANSEN, THEODORE L.
OFFUTT, EDWARD P.
PIMENTEL, DAVID
PORTUONDO, BONAVENTURE CHARLES
SHELDON, JOHN FISK
STEINER, HENRY
IMBODEN, CLARENCE ALPHONSE, JR.
KNUTSON, HERBERT
LANG, FREDERICK ROBERT
LELAND, STANLEY JOHNSON
MAXWELL, ELMER S.
MORGAN, EDWARD H.
MUNCH, JAMES C.
TANNER, RONDELL H.
TAYLOR, FRANK CHESSROWN
VON POHLE, CHARLES LAWRENCE
WARE, EDWARD RICHMOND

The following, delinquent in dues for 1953, 1954, and 1955, were dropped from the rolls on January 1, 1956 (as per ARTICLE XI of the BY-LAWS):

BEIN, SOLOMON S.
BRUHL, CHARLES KENNEDY
CLAYMAN, CHARLES B.
FELLTON, HERMAN L.
GALLARDE-C, MARCELO
GOMEZ-R, GILBERTO
GONZALES-MUGABURU, LUIS
HARTMAN, ERNEST
HAYES, HAROLD B.
HOCKWALD, ROBERT S.
KADER-M, MOHAMED ABDEL
KIRBY, A. J.
KOSERUBA, GEORGE MICHAEL
LAUBLY, CHARLES S.
LIKOS, JOSEPH J.
LOPEZ-FERNANDEZ, FERNANDO
MCCAULEY, W. E.
MARTIN, JAMES R.
MONTERO, ENRIQUE
RAMSAY, SAMUEL WATSON
SHORTT, HENRY EDWARD
SOBTI, S. K.
VIRANUVATTI, VIKIT
WOOD, FRANK B.

(9) *Letters to the Officers and Councilors.*—Five letters were sent during the period of this report. The first, February 9, concerned general plans for the New Orleans meeting. It was decided, by a majority vote, to follow the pattern of the Boston program, to have one symposium (see above), and to accept tentatively the dates of October 30–November 2 for the *1957* meeting. On March 8, the Council voted to renew for 3-years the present publishing agreement for our JOURNAL (see above). On June 4, the Council voted unanimously in favor of the nomination (Dr. Karl F. Meyer) presented by the Walter Reed Award Committee, expressed the desire to have a slate of candidates before voting for the new Secretary-Treasurer, voted to accept the date of December 31, 1956 as the retirement date of the present Secretary-Treasurer, voted to authorize expenses for the in-coming Secretary-Treasurer to cover an orientation trip to Chapel Hill, and voted to invite the in-coming Officer to the Council meeting in New Orleans. On June 25, the Council voted in favor of a proposal to hold the 1958 World Health Assembly of the WHO in the United States thereby authorizing a resolution to this effect to be sent to the appropriate members of Congress, and elected Dr. Rolla B. Hill as Secretary-Treasurer, effective January 1, 1957. On September 26, the fifth and final letter was sent. This concerned a reminder of the scheduling of the Council meetings in New Orleans and a request to send items for the agenda before October 15.

(10) *Other Activities.*—Those in connection with various committees will be presented below under the appropriate headings.

Finally, the splendid cooperation of the Officers, Councilors, members of committees and others in transacting the business of the Society during my four years in office is hereby acknowledged with sincere thanks and appreciation.

Respectfully submitted,
JOHN E. LARSH, JR.
Secretary

The report of the Secretary was accepted as read with an expression of appreciation by the Council.

The report of the Editor of the AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE was presented as follows:

This has been a satisfactory year for the Journal, financially and otherwise. The situation as of September 30 is set forth in Table 1. At the instance of the auditors, the period of this report has now been shifted to cover the same financial year as that of the Society—October to September—so that the figures may be brought more nearly up to date for the benefit of the annual meeting. There seems to have been no good reason for limiting the accounts to the previous calendar year as has been done heretofore.

There was a good margin of income over production cost, half of which, or about $2,300, accrued to the Society. This however is not pure gain and hence subject to appropriation as the Council has sometimes supposed, since out of it must be paid the expense of maintaining the editorial office, which is not shown in the table and amounted to about $1,900. Thus available surplus is actually only some $400, which will probably all be needed to supplement the income from the Society's invested funds, earmarked for but insufficient to pay the cost of publication of abstracts of papers presented at the annual meetings, and of one or more review articles which it is hoped can be presented every year in the Journal. (Two of these reviews, "Malaria," and "Sanitation in the Tropics," will appear shortly.) Our income and expenses do not vary much from year to year and are liable to be upset only by a significant increase in publication costs.

Table 1 also shows that the Journal was able to print during the year 262 pages more than the 900 pages contracted for with the publishers. These were paid for in various ways—by institutions and authors interested in the prompt publication of their papers; by the Society itself for its own purposes as explained above; and by the "Journal fund," set up some years ago to permit publication of more than the contract number of pages whenever

TABLE 1

Financial Statement, American Journal of Tropical Medicine and Hygiene, for one year—covering 6 issues: V. 4, n. 5 (Sept. 1955) to V. 5, n. 4 (July 1956)

	Vol. 3 (1954)	Vol. 4 (4 issues, 1955) Jan.–July	Vol. 4–5 (6 issues, from Sept. 1955–July 1956)
Income:			
Paid subscriptions	$18,613.42	$12,325.65	$18,580.00
Credit for pages unused in previous yr	620.18	—	—
Total from subscriptions	19,233.60	12,325.65	18,580.00
Space sales for advertising	2,821.58	1,490.86	2,060.00
Paid by contributors for extra pages	2,183.24	2,736.32	2,642.12
Net from reprint sales	627.38	549.57	1,007.98
Back volume sales	590.31	321.27	445.00
Total income	25,456.12	17,423.67	24,735.10
Production cost:			
Printing of text	15,410.50	10,152.61	15,356.12
Printing advertisements	891.61	421.40	667.00
Distribution, overhead, etc.	4,151.67	2,826.16	4,095.00
Total cost	$20,453.78	$13,400.17	$20,118.12
Surplus (income less cost)	5,002.34	4,023.50	4,616.98
Amount due ASTMH (50%)	2,501.17	2,011.75	2,308.49
Circulation and size:			
No. of paid subscriptions (members)	1,088	1,078	1,029
No. of paid subscriptions (nonmembers)	1,265	1,199	1,256
Total paid subscriptions	2,353	2,277	2,285
Pages			
text	1,128	768	1,162
advertising	80	46	65
Total pages	1,208	814	1,227
Average cost of printing a page of text	$13.66	$13.22	$13.22

the necessity should arise. Such funds do not contribute to our surplus, since they are expended in paying for printing the articles, but they determine the size of each number, and last year provided generous issues averaging 195 pages.

Table 2 shows the flow of manuscripts in 1955; we received 164, accepted 127 (77%), and were able to publish 125, an average of 21 per issue. These papers when printed averaged 8½ pages in length. At the end of the year we had 84 unpublished manuscripts to carry forward to 1956, enough to make up four average numbers. This means that unsubventioned papers submitted in the early months of 1956 had to wait nine or ten months for publication, an unsatisfactory situation. The outlook is now more favorable to authors. The backlog of unpublished manuscripts has been reduced considerably, and it is hoped that the lag between the receipt of a manuscript and its appearance in the Journal can be shortened to about six months next year. This is not unreasonable, since it frequently takes that long for the Editor and the author to come to agreement on what is to be published.

Table 3 shows the fields covered by papers published during the last two years. They followed the established pattern fairly well, but there was a general increase in papers in

TABLE 2

Number and disposal of manuscripts submitted

Manuscript	Vol. 3 (1954)		Vol. 4 (1955)	
From previous year		90		82
Submitted during year	*147*		*164*	
Declined	21		34	
Withdrawn	6		3	
Accepted		*120*		*127*
Available for publication		210		209
Published during year		*128*		*125*
On hand for next volume		82		84

TABLE 3

Subjects	Average 1952–1955	1956	Approach	Average 1952–1955	1956
Animal hosts & vectors	25	30	Biology—taxonomy	6	5
Bacteria & fungi	11	15	Clinical (diagnosis, treatment)	34	33
Deficiencies (nutrition, etc.)	1	1	Epidemiology & statistics	30	39
Helminths	22	27	Experimental—Field	3	2
Protozoa	44	29	Experimental—Lab.	29	32
Viruses & Rickettsias	12	21	Prevention & Control	13	9
General	6	2	Others	6	5
Total	121	125	Total	121	125

all subjects except Protozoa, which fell off rather badly. Considering the small numbers involved, this probably has no significance. Virtually no papers on nutrition in the tropics are submitted to our Journal. Since our Journal pretends to be an international medium, I am happy to report that we received manuscripts in 1956 from nationals or long-time residents of the following 21 countries: Belgium, Belgian Congo, Egypt, Guatemala, Honduras, India, Indonesia, Israel, Italy, Japan, Lebanon, Liberia, Netherlands New Guinea, Portuguese Guinea, Puerto Rico, Saudi Arabia, Taiwan, Thailand, Uganda, the United States, and Venezuela.

Respectfully submitted,
L. W. HACKETT
Editor

The Editor's report was accepted as read with an expression of sincere appreciation by the Council.

The report of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS was read as follows by the Secretary-Treasurer in the absence of the Editor:

Responsibility for editing the "News" was transferred to the present editor on January 1, 1956. No important changes were made in policy or management.

Important support was given to the "News" by Chas. Pfizer and Company, Burroughs-Wellcome and Company, and Winthrop Laboratories who continued to advertise in our pages. The American Foundation for Tropical Medicine has also continued to support the

"News" by defraying part of the cost of each issue in return for distribution to their mailing list.

"Health Hints for the Tropics" has continued in great demand. In the early months of the year the booklet was reviewed and revised with the help of numerous members and friends of the Society. In May the revised edition, incorporating many changes but retaining the same general subject matter, was printed in 13,000 copies. The Pan-American Sanitary Bureau and the American Foundation for Tropical Medicine both contracted for 4,000 copies in advance thus making possible a considerable saving on printing costs. A reprinting will be ordered soon.

Plans have been made to print the Society Membership List in a separate booklet to be mailed with the December issue of the "News."

Advertising revenue has decreased in recent years and costs have risen slightly. As in the preceding two years, costs of production of the "News" have nearly balanced income. Somewhat increased income from advertising is needed if the "News" is to continue to be self-supporting in the face of increasing costs.

Respectfully submitted,
LOUIS J. OLIVIER
Editor

This report was accepted as read whereupon discussions were held concerning two problems presented by the Editor in letters covering his report. The first in regard to the need for assistance in securing additional advertisers for the NEWS, resulted in a motion that was carried authorizing the in-coming President to appoint an individual or committee to assist the Editor in this area of operations. The other problem, concerning a different price schedule in use by the two offices selling "Health Hints for the Tropics" (the Editor's office, and that of the American Foundation for Tropical Medicine), was discussed at length, after which the following motion was entered and carried: "that both offices shall continue to sell the "Hints," and beginning January 1, 1957, the price schedule for both offices shall be that in current use by the Foundation, and that a notice to this effect shall be published in the December, 1956 issue of the NEWS."

The Treasurer's report was presented as follows:

October 23, 1956

The Officers and Councilors
The American Society of
Tropical Medicine and Hygiene
Chapel Hill, North Carolina
Gentlemen:

The Balance Sheets at September 30, 1956, and the Statements of Cash Receipts and Disbursements for the year ended September 30, 1956, of The American Society of Tropical Medicine and Hygiene and of the *Tropical Medicine and Hygiene News*, have been reviewed and found in order.

The cash receipts and disbursements were traced through the bank accounts. Cash in banks were confirmed directly from the Bank of Chapel Hill, Chapel Hill, North Carolina, and the Bank of Bethesda, Bethesda, Maryland.

U. S. Savings bonds in the total amount of $20,198.00, stated at cost, were inspected and found to be in the name of the Society.

The amount due from The Williams & Wilkins Company, publishers of *The Journal*, represents the estimated profits for the publication of the first four issues in 1956. This amount was confirmed directly from the publishers.

As to membership dues, it was not practicable to extend the review beyond that receipts as reported on the books had been deposited in the Society's bank account. The following is a comparative summary of the membership dues collected and payments to the publishers for subscriptions to *The Journal*:

	Year Ended Sept. 30, 1956	*Year Ended Sept. 30, 1955*
Dues Collected:		
Prepayments	$ 60.44	$ 52.00
Current Year	8,426.00	8,436.62
Prior Years	175.78	242.00
Total Dues Collected—Exhibit B	$8,662.22	$8,730.62
Payments to Publishers—Exhibit B	6,532.00	6,630.00
Net Increase in Dues, Exclusive of Subscriptions to *The Journal*	$2,130.22	$2,100.62

The investment in *Tropical Medicine and Hygiene News*, Exhibit A, shows an increase of $415.13 over the preceding year. Although, in Exhibit D, receipts from advertising decreased $75.00, sales of supplements increased $913.28 and the receipts from American Foundation for Tropical Medicine increased $194.57 over the previous year. The value of the inventory of "Health Hints for the Tropics" is at estimated cost of $222.00 and has not been included in the Balance Sheet at September 30, 1956, Exhibit C, nor in the investment of The Society in *Tropical Medicine and Hygiene News* in Exhibit A. This inventory was not counted or confirmed by correspondence.

The cash receipts of The Society, Exhibit B, were approximately the same as the previous year, with the exception that The Society received $1,000.00 from *Tropical Medicine and Hygiene News* in 1955. There was no transfer of funds from the publication of the *News* to The Society during the current period under review. Operating expenses of the editor and the secretary-treasurer are comparable to the previous year, with the exception that the year ended September 30, 1955, included the expenses of the editor for a 21-month period instead of the usual twelve months.

Attached to this letter are the following statements presented in comparative form with those of the preceding year:

	Exhibits
The American Society of Tropical Medicine and Hygiene, Chapel Hill, North Carolina:	
Balance Sheets, at September 30, 1956 and 1955	A
Cash Receipts and Disbursements, Years Ended September 30, 1956 and 1955	B
Tropical Medicine and Hygiene News, Bethesda, Maryland:	
Balance Sheets, at September 30, 1956 and 1955	C
Cash Receipts and Disbursements, Years Ended September 30, 1956 and 1955	D
	Schedules
The American Society of Tropical Medicine and Hygiene, Chapel Hill, North Carolina:	
Investments in U. S. Savings Bonds, at September 30, 1956 and 1955—Exhibit A	1
Office Expenses, Years Ended September 30, 1956 and 1955—Exhibit B	1

Yours truly,
(Signed)
ALTON G. SADLER, C.P.A.

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

BALANCE SHEETS

September 30, 1956 and 1955 *Exhibit A*

	September 30, 1956		*September 30, 1955*	
Assets				
Current Assets:				
Cash In Bank and On Hand—Exhibit B		$2,213.32		$2,009.66
The Williams & Wilkins Co.—Note 1		1,513.32		2,011.75
Interest Receivable, U. S. Savings Bonds—Schedule 1		179.40		179.40
Total Current Assets		3,906.04		4,200.81
Investments:				
U. S. Savings Bonds, at Cost, Note 2—Schedule 1:				
Series J, Maturity Value $275.00	$198.00		$198.00	
Series K, 2.76%	20,000.00		20,000.00	
Total U. S. Savings Bonds	20,198.00		20,198.00	
Tropical Medicine and Hygiene News, Note 3—Exhibit C	1,610.51	21,808.51	1,195.38	21,393.38
Office Equipment		472.24		472.24
Total Assets		$26,186.79		$26,066.43
Liabilities and Net Worth				
Prepaid Dues		$60.44		$52.00
Net Worth:				
Retained Earnings—Note 4				
Balances, September 30, 1955 and 1954	$25,542.19		$24,611.86	
Increase for Years Ended September 30, 1956 and 1955	111.92		930.33	
Balances, September 30, 1956 and 1955	25,654.11		25,542.19	
Investment in Office Equipment	472.24	26,126.35	472.24	26,014.43
Total Liabilities, and Net Worth		$26,186.79		$26,066.43

Notes:

1. The account receivable from The Williams & Wilkins Company represents the estimated profit for the publication of four issues of *The American Journal of Tropical Medicine and Hygiene*.

2. Interest on U. S. Savings Bonds, Series J, is available for current expenditures; interest on all Series K Bonds is available for reviews in *The Journal* and for publishing abstracts of papers on the program.

3. Inventory of "Health Hints for the Tropics," at estimated cost of $222.00 is not included in the invested amount $1,610.51.

4. Of the $25,654.11 in Retained Earnings at September 30, 1956, $2,650.40 is unappropriated at September 30, 1956, the remainder having been appropriated for:

Extra pages for *The Journal*	$1,904.38
Life Membership Fee of Dr. Charles A. Werner invested in U. S. Savings Bonds, Series J	198.00
Investment in U. S. Savings Bonds, Series K, the interest on which is available for reviews for *The Journal* and for publishing abstracts of papers on the program	20,000.00
Interest on Series K Bonds, Collected to Sept. 30, 1956, $1,159.20, less cost of publishing 17 pages of abstracts of the 1955 Boston Meeting, $257.87	901.33
Total Appropriated Retained Earnings	$23,003.71

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

INVESTMENT IN U. S. SAVINGS BONDS — *Exhibit A*

September 30, 1956 and 1955 — *Schedule 1*

Serial Number	*Date of Purchase*	*Cost*	*Maturity Value*	*Accrued Interest*
Series J:				
Q 21,413J	6-29-54	$18.00	$25.00	
Q 21,414J	6-29-54	18.00	25.00	
Q 21,415J	6-29-54	18.00	25.00	
Q 93,631J	6-29-54	72.00	100.00	
Q 93,632J	6-29-54	72.00	100.00	
Total Value of Series J		$198.00	$275.00	
Series K:				
M 91,038K	4-14-53	$1 000.00	$1 000.00	$13.80
M 91,039K	4-14-53	1 000.00	1 000.00	13.80
M 91,040K	4-14-53	1,000.00	1,000.00	13.80
M 91,041K	4-14-53	1,000.00	1,000.00	13.80
M 195,142K	6-29-54	1,000.00	1,000.00	9.20
V 26,490K	6-29-54	5,000.00	5,000.00	46.00
V 26,514K	7-9-54	5,000.00	5,000.00	34.50
V 26,515K	7-9-54	5,000.00	5,000.00	34.50
Total Value of Series K 2.76%		$20,000.00	$20,000.00	$179.40
Total Value of Investments		$20,198.00	$20,275.00	$179.40

Notes:

There were no purchases or sales of U. S. Savings Bonds in the year ended September 30, 1956.

All bonds are in the name of The American Society of Tropical Medicine and Hygiene, a corporation, in care of School of Public Health, Chapel Hill, North Carolina.

The Series J Bonds were purchased with funds from the life membership of Dr. Charles A. Werner. The interest therefrom is available for current expenditures. Interest accrues to the value of these bonds periodically according to the length of time held, and the realizable values at September 30, 1956, were $204.05 and at September 30, 1955, were $200.46.

The Series K Bonds were purchased in 1954 with funds from current operations $6,027.50, reinvestment of $2,016.00 Series J redeemed for $2,058.00, and reinvestment of $6,512.00 Series F from the dissolved American Society of Tropical Medicine redeemed for $7,914.50. Interest on all Series K Bonds is available for reviews in The Journal and for publishing abstracts of papers on the program.

Interest on Series K Bonds to September 30, 1956, is summarized as:	
Collections for the fiscal years ended September 30, 1954, 1955, and 1956	$1,159.20
Less Cost of publishing 17 pages of abstracts of 1955 Boston Meeting as part of 1955 transactions (see The Williams & Wilkins Co. invoice number 41,103, dated May 21, 1956)	257.87
Total interest available	$901.33

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

Years Ended September 30, 1956 and 1955 — *Exhibit B*

		Years Ended September 30 1956		1955
Cash Balances, Sept. 30, 1955 and 1954		$2,009.66		$2,245.17
Receipts:				
Membership Dues:				
1957	$60.44		—	
1956	8,426.00		$52.00	
1955	149.78		8,436.62	
Prior Years	26.00	8,662.22	242.00	8,730.62
The American Journal of Tropical Medicine & Hygiene Profit for Calendar Years 1955 and 1954		2,788.45		2,501.17
Publication of *Tropical Medicine and Hygiene News*—Exhibit C		—		1,000.00
Annual Meetings at Boston, Mass., 1955 and Memphis, Tenn., 1954:				
Share of Income from Exhibits	150.69		361.30	
Registrations	259.60		486.30	
Refunds on Advances to Local Committees on Arrangements	50.00	460.29	100.00	947.60
Interest on U. S. Savings Bonds		552.00		552.00
Burroughs-Wellcome Co. Contribution for New Orleans Meeting		300.00		—
Commemorative Medal Sales		49.50		107.00
American Academy of Tropical Medicine, Balance in Bank		—		90.40
Sale of Back Issues of *The Journal*		—		12.00
Total Receipts		$12,812.46		$13,940.79
Total Available Cash		$14,822.12		$16,185.96
Disbursements:				
Office Expenses—Schedule 1:				
Editorial	1,912.34		1,923.11	
Secretary-Treasurer	3,606.59		5,123.19	
Total Office Expenses	5,518.93		7,046.30	
Burroughs-Wellcome Co. Contribution for New Orleans Meeting	300.00		—	
Subscriptions to *The Journal*	6,532.00		6,630.00	
Publication of Abstracts—Exhibit A, Schedule 1	257.87		—	
Le Prince Award	—		500.00	
Total Disbursements		12,608.80		14,176.30

Cash Balances, Sept. 30, 1956 and 1955—Exhibit A..	$2,213.32	$2,009.66
Consisting of:		
Cash in Bank	$2,188.86	$2,003.89
Cash on Hand	24.46	5.77
Total Cash in Bank and on Hand—Exhibit A....	$2,213.32	$2,009.66

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

OFFICE EXPENSES — *Exhibit B*

Years Ended September 10, 1956 and 1955 — *Schedule 1*

	Years Ended September 30			
	1956		*1955*	
Accounts	*Editorial*	*Secretary Treasurer*	*Editorial*	*Secretary Treasurer*
Secretarial Assistance	$693.37	$2,400.00	$1,144.88	$2,600.00
Honorarium, Assistant to Editor	787.50	—	187.50	—
Travel and Meetings	243.10	316.15	307.34	284.72
Office Equipment	—	—	—	32.66
Postage	121.70	286.90	177.00	401.76
Printing	32.29	214.40	51.00	653.34
Office Supplies	17.60	54.48	44.94	76.36
Telephone and Telegraph	16.78	2.97	10.45	14.39
Auditing Fee	—	175.00	—	125.00
Dies for Medals	—	—	—	600.75
Medals	—	17.50	—	192.37
Advance for National Meetings	—	—	—	50.00
Freight and Express	—	32.20	—	36.84
Corporate Registration	—	30.00	—	30.00
Contribution to National Society for Medical Research	—	25.00	—	25.00
Insurance	—	51.99	—	—
Total Office Expenses	$1,912.34	$3,606.59	$1,923.11*	$5,123.19

* Includes expenses from January 1, 1954, through September 30, 1955.

Tropical Medicine and Hygiene News
Bethesda, Maryland

BALANCE SHEETS

September 30, 1956 and 1955 — *Exhibit C*

Assets

	September 30, 1956	*September 30, 1955*
Current Assets:		
Cash in Bank and on Hand—Exhibit D	$1,438.99	$946.50
Accounts Receivable	124.05	212.69
Stamps and Postage Deposit on Metered Mail	47.47	36.19
Total Assets	$1,610.51	$1,195.38

Net Worth		
Retained Earnings:		
Balances, Sept. 30, 1955 and 1954	$1,195.38	$1,989.29
Increase for Years Ended Sept. 30, 1956 and 1955	415.13	206.09
	1,610.51	2,195.38
Payment to The American Society of Tropical Medicine and Hygiene—Exhibits B and D	—	1,000.00
Total Net Worth—Exhibit A	$1,610.51	$1,195.38

Note: Inventory of "Health Hints for the Tropics" at estimated cost of $222.00 is not included in this balance sheet at September 30, 1956.

STATEMENTS OF CASH RECEIPTS AND DISBURSEMENTS

Years Ended September 30, 1956 and 1955 *Exhibit D*

	Years Ended September 30			
	1956		1955	
Cash Balances, Sept. 30, 1955 and 1954		$946.50		$1,833.27
Receipts:				
Advertising	$1,500.00		$1,575.00	
American Foundation for Tropical Medicine	623.98		429.41	
Sale of Supplements	1,144.95	3,268.93	231.67	2,236.08
Total Available Cash		4,215.43		4,069.35
Disbursements:				
The American Society of Tropical Medicine and Hygiene—Exhibits B and C	—		1,000.00	
Printing of *News*	978.52		838.20	
Printing of "Health Hints"	700.64		—	
Copy Preparation	472.90		399.90	
Secretarial Assistance	172.90		300.00	
Office Supplies	29.89		158.74	
Travel	127.46		162.19	
Postage and Express	155.42		133.39	
Postage Meter Permit Deposits	130.00		100.00	
Miscellaneous	9.61	2,776.44	30.43	3,122.85
Cash Balances, Sept. 30, 1956 and 1955		$1,438.99		$946.50
Consisting of:				
Cash in Bank of Bethesda, Bethesda, Md.		$1,434.63		$944.32
Cash on Hand		4.36		2.18
Total Cash on Hand and in Bank—Exhibit C		$1,438.99		$946.50

As noted above the present report, covering the period from October 1, 1955 to October 1, 1956, includes a direct comparison with last year's report. Due to necessary changes in the auditing system from year to year after the formation of the new Society, it has not been possible previously to make this comparison directly. Especially for the benefit of the Council in determining financial policies, it would seem desirable to include this direct comparison in all subsequent reports.

As shown on the balance sheets (Exhibit A), the total assets, total liabilities, and net worth were about the same at the closing of the financial books in 1955 and 1956. Also, the receipts and disbursements for the NEWS were about the same for 1955 and 1956 (Exhibit D). Thus, for next year we should be able to predict accurately the financial outlay necessary to operate the offices of the Secretary-Treasurer, the Editor of the JOURNAL, and the Editor of the NEWS. The major receipts making it possible to meet the necessary expenses of the Society have been the membership dues, the profit on the JOURNAL, the profit on the NEWS, and the interest on our investments (Exhibit B). This year it was not possible to transfer to the treasury any funds from the News account (see Editor's report above). Without the usual $1,000.00 from the NEWS, we ended the year with a balance in the treasury of $2,213.32. It is important to point out that this can not be considered as surplus, since it is less than the amount ear-marked for two appropriated items (extra pages in the JOURNAL [$1,904.38], and reviews and abstracts [$901.33]—see Exhibit A). To cover the two appropriated items, we should have ended the year with a balance of $2,805.71, so that we were short by $592.39. Therefore, without the transfer of funds from the NEWS we were not able to balance the books. This, however, is more apparent than real, in that the estimated profit on the 4 issues of the JOURNAL for 1956 ($1,513.32—see Exhibit A) covers the deficit and leaves a balance of about $1,000.00. Nevertheless, it would seem wise to increase our receipts by about $500.00 to bring about a balance in our books without considering the profits on the JOURNAL for the current year. Perhaps the best method for doing this is to increase the advertising revenue for the NEWS as suggested by its Editor (see above). The other alternative, of course, is to reduce our expenditures by $500.00, but in the eyes of the present Officer this would not be practical in the near future.

It is obvious from the above that great care must continue to be exercised to avoid deficit spending, and to maintain our investments. Since the latter are providing interest to further the objectives of the Society every effort should be made to retain them and, if possible, to increase them.

Recommendations.—(1) that the present date (September 30) for closing the financial books, and the present system of reporting our financial status remain the same for next year, (2) that during the next year the Secretary-Treasurer be bonded for $25,000 (ARTICLE IV of the By-laws), and (3) that the Secretary-Treasurer be authorized during the next year to spend monthly for secretarial assistance a sum not to exceed $200.00.

Respectfully submitted,
JOHN E. LARSH, JR.
Treasurer

The Treasurer's report was accepted as read, and the three recommendations were approved as listed.

*Reports of Committees**

Bailey K. Ashford Award. Submitted by W. A. Sodeman

The Committee, after voting against presenting the Award this year, agreed to suggest to the Council that the Committee be activated next year. The Council approved the activation of the Committee for 1956–57, and authorized the Committee to consult with the sponsor regarding the possibility of raising the age limit. The findings of the Committee are to be referred to the Council for final action. As a further means of increasing the number of nominations for this attractive Award, the Council suggested that the Committee take a more active role in having qualified individuals nominated by the membership.

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* For a complete list of the committees activated this year, see the February, 1956 issue of the NEWS.

† *Development and Needs.* Submitted by A. B. Sabin

The National Research Council is pursuing its task (see 1955 Transactions) and is currently awaiting word from a private foundation regarding an application for funds.

† *Examination of Fecal Specimens for Intestinal Parasites.* Submitted by H. E. Meleney

The Committee recommends that the Society continue to stimulate accurate fecal examination for parasites by the following activities: 1) Urge physicians to check the proficiency of the laboratories they utilize (including their own) by periodic national or state parasitological evaluation. 2) Urge the establishment of continuous evaluation programs (national and state). Medical and technological societies should be encouraged to request this type of service from public health laboratories. 3) Urge the employment of professional parasitologists by state laboratories. The parasitologists could supervise the diagnostic activities of the state laboratory, direct intrastate evaluation programs and conduct local training courses throughout the state. 4) Urge boards which are responsible for the certification of medical technologists to emphasize the identification of intestinal protozoa, both in training courses and in qualifying examinations. 5) Urge the establishment of permanent distributing centers for parasitological specimens, both for teaching and evaluation, to be made available to federal, state and local hospitals and laboratories, private hospitals and laboratories, and educational institutions.

Membership. Submitted by M. M. Brooke

The Committee approved 62 applications for membership. The total membership is 1217: 16 honorary members, 7 emeritus members, one life member, and 1193 active members (of which 1074 are in good standing and 119 are delinquent in dues). In keeping with the feeling expressed by the Council during the Memphis meeting, no attempt was made to solicit new members.

Program. Submitted by J. E. Larsh, Jr.

In mid-January, the Committee agreed on general plans for the New Orleans meeting, including a tentative program summary. On February 9, the Council approved the recommendations of the Committee, and selected N. R. Stoll as Moderator for the symposium on "Helminthic Infections as the Cause of Disability and Disease." It was then possible to proceed with specific plans for the meeting.

All members of the Society were sent an announcement of the meeting on March 27, 1956, and were requested to submit before July 1 applications and abstracts for papers and/or scientific exhibits. Seventy-eight requests to read a paper and 6 requests to mount a scientific exhibit were received. On July 17, the materials were forwarded to the members of the Committee for review and action. By a majority vote, it was decided to accept all of the applications, limiting the time of each paper to 10 minutes. To accommodate the unprecedented number of papers, it was necessary to schedule a double scientific session for Thursday morning, November 1. Those who submitted applications were notified of the Committee's action on August 6, and a copy of the printed program was sent to all members of the Society on September 25.

† *Public Relations.* Submitted by G. M. Saunders

At the annual meeting of the Society in 1955 it was voted to create a Committee on Public Relations. The stated objectives of the Committee are to promote greater interest, particu-

† *Ad hoc* Committee

larly in Congress, in tropical medicine, parasitology and microbiology, and to provide information to budget committees on the importance of tropical medicine to our country and the need for support for research and teaching in these fields. The Committee was formed early in January 1956 when your President appointed a chairman and two members.

Although the Committee has not met as a group, there have been frequent communications between the members by telephone and by letter. Close working arrangements were established with the Public Relations Committee of the American Society of Parasitologists through the chairman, Dr. Gilbert Otto, and with Dr. Walsh McDermott of the National Advisory Health Council.

Letters were sent to the Honorable Lister Hill, chairman of the Senate Committee on Labor and Public Welfare, and to the Honorable John E. Fogarty, chairman of the Labor and Health, Education and Welfare Subcommittee of the House Committee on Appropriations. These letters, from our Committee on Public Relations, stated the position of our Society urging careful consideration of adequate support for infectious disease research and offering to testify before appropriate hearings of the Senate and House. On Tuesday, May 8, 1956, Dr. Tom F. Whayne testified before the Committee on Labor and Public Welfare of the Senate. A letter of 26 March 1956 was sent to the Honorable Lister Hill supporting the views put before his Committee of the Senate by Dr. Walsh McDermott on 28 March 1956 in a formal statement.

During the past five years funds available for extramural grants through the Allergy and Infectious Diseases Institute amounted to $2,110,000.00 per year. This year the budget proposal was for $4,950,000.00 but this amount was cut by the House Committee by $1,000,000.00. However, the Senate restored this cut and increased the total amount to $8,065,000.00. This amount was subsequently supported by Congressman Fogarty in a bill introduced and passed by the House on 27 June 1956.

It is felt that your Committee through its activities may have had some influence in the action of Congress which provided increased funds for allergy and infectious disease research.

All of the above reports were accepted by the Council with appreciation for the efforts of the various committee members. It was voted to continue for another year two of the *ad hoc* Committees, Development and Needs (with the same members that served this year), and Public Relations.

Reports of Representatives to Organizations

A.A.A.S. Submitted by E. E. Byrd and R. E. Dyer

Portions of the report of the two meetings of the A.A.A.S. Council (Atlanta, Georgia, December 27 and 30, 1955) dealing with matters of most concern to our Society were read.

American Foundation for Tropical Medicine. Submitted by E. I. Salisbury

A report was given summarizing the activities during the year of the Foundation and of the Liberian Institute of the Foundation.

These various reports were accepted as read.

Other Old Business

Election of Officers and Councilors for 1956–57.—The Nominating Committee (E. H. Hinman, Chairman; L. E. Rozeboom, and H. W. Van Hovenberg) submitted a slate, which was mailed with a ballot to all voting members of the Soci-

ety. The votes were tabulated by the Tellers, W. W. Cort and J. R. Hendricks, appointed by the President. The results of the election were as follows:

President-Elect	D. L. AUGUSTINE
Vice-President	G. R. COATNEY
Councilors	J. M. HENDERSON
	J. A. SCOTT

New Business

Committee Appointments.—In addition to the appointment of Tellers for this meeting (see above), the President appointed J. M. Henderson (Chairman), M. M. Brooke, and H. F. Schoof as members of the Resolutions Committee.

The Council elected the following members to serve on the Nominating Committee for 1956–57: F. J. Brady (Chairman), L. B. Hall, and J. C. Swartzwelder. The Council also elected W. W. Frye to serve on the Bailey K. Ashford Award Committee during 1957, 1959, and 1961.

Contribution to the National Society for Medical Research.—After a discussion of the program of this organization, a contribution of $25.00 was voted.

Report on Work of the International Interim Committee, Sixth Congresses of Tropical Medicine and Malaria.—F. J. Brady, Past-President of the Society and one of the two members on the Committee from North America, gave a brief report on the preparations for the Congresses. It is planned to publish part of this report in an early issue of the NEWS.

Observations Regarding Certain Aspects of Work in Tropical Medicine in the Soviet Union.—A. B. Sabin reported on certain of his observations made on a recent visit.

1957 Meeting.—The Secretary-Treasurer reported that he had received a fully documented invitation from the city of Philadelphia and various educational institutions located there to hold the meeting in Philadelphia. After inspecting the facilities, and in consideration of various important factors (adequate number of local members to form a local committee, etc.), the Secretary-Treasurer recommended Philadelphia as the 1957 meeting site. The Council approved this recommendation, and the dates of October 30–November 2 were selected. When it was announced that the Council of the American Society of Parasitologists had expressed an interest in a joint meeting, the Secretary was instructed to invite them to join us.

Ad Hoc Committees for 1956–57.—The Council authorized the President to appoint such committees that he and the Secretary-Treasurer deem necessary.

Election of FELLOWS.—Using as a guide the nominations submitted by the membership on the ballot for the election of Officers and Councilors (sent March 27, 1956), the Council elected the following members to the status of FELLOW: R. Elsdon-Dew, A. R. Neghme, J. Singh, K. C. Smithburn, and L. Van Den Berghe. The in-coming Secretary-Treasurer, R. B. Hill, becomes a FELLOW automatically as per the provisions adopted last year by the Council.

MINUTES OF THE ANNUAL BUSINESS MEETING

The annual business meeting of the Society was held November 3, 1956 at 8:30 A.M. in the Tulane Room of the Jung Hotel, New Orleans, Louisiana, with President Asa C. Chandler presiding.

The minutes of the fourth meeting (Boston, Massachusetts, November 5, 1955) were accepted as published in the JOURNAL, March, 1956.

The Secretary-Treasurer's reports were approved as read.

The Editor-in-Chief of Publications, L. W. Hackett, presented the Journal report, which was accepted as read with a vote of thanks being extended to the Editor.

The report of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS, Louis J. Olivier, was read by the Secretary-Treasurer in the absence of the Editor. This report was accepted with a vote of thanks to the Editor.

Reports of Committees and Representatives.—A summary of certain of the reports presented at the Council meetings (see above) was presented by the Secretary-Treasurer. These reports were approved by a voice vote.

Announcement Regarding Council Action on Old Business.—The results of the election of Officers and Councilors by mail ballot were read by the Secretary-Treasurer. D. L. Augustine was elected President-Elect for 1956–57, G. R. Coatney was elected Vice-President, and J. M. Henderson and J. A. Scott were elected Councilors for four years.

Announcements Regarding Council Action on New Business.—The Secretary-Treasurer read the following announcements: (1) that the Council had appointed F. J. Brady (Chairman), L. B. Hall, and J. C. Swartzwelder to serve on the 1957 Nominating Committee, and W. W. Frye to serve on the Bailey K. Ashford Award Committee (1957, 1959, 1961), (2) that a $25.00 contribution had been voted for the National Society for Medical Research, and (3) that six active members had been elected FELLOWS of the Society using as a guide the list of nominations submitted by the membership (the names to be published in an early issue of the NEWS). Finally, it was announced that the Council had accepted the documented invitation to hold the 1957 annual meeting in Philadelphia. The meeting will be held October 30–November 2, 1957, and the American Society of Parasitologists will be invited to meet jointly with us.

Report of the Resolutions Committee (J. M. Henderson, Chairman; M. M. Brooke; and H. F. Schoof)

I. It is requested that the Secretary of the Society be instructed to write and send resolutions of sympathy to the families of deceased fellows and active members of the American Society of Tropical Medicine and Hygiene. During the current year, the Society has lost 3 pioneers and outstanding leaders, namely William H. W. Komp, Fellow; Joseph A. A. LePrince, Fellow; and William O. French, Member.

II. To Dr. Norman R. Stoll, Moderator, and members of his panel, the thanks and appreciation of the Society are extended for the outstanding and enlightening symposium on the Helminthic Infections as the Cause of Disability and Disease.

III. To Dr. Leon H. Schmidt, we extend this Society's sincere thanks and highest esteem for his able presentation of the 21st Annual Charles Franklin Craig Lecture, *"The Role of Pharmacology in Determining the Rational Use of Therapeutic Agents."*

IV. To the organizations and individuals who have served as hosts for the Fifth Annual Meeting of the Society held in New Orleans, Louisiana, October 31–November 3, 1956, or otherwise furnished helpful assistance:

A. The Officers and Members of the Society are deeply appreciative of the assistance and hospitality extended by the Schools of Medicine of Louisiana State University and Tulane University of Louisiana, members of the Faculties and staffs. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to Dr. William W. Frye, Dean, and Dr. Maxwell E. Lapham, Dean, and through them to their respective institutions, Faculties and staffs.

B. The Chamber of Commerce of the New Orleans Area has provided secretarial help and other assistance and has otherwise contributed greatly to the success of the Meeting. THEREFORE BE IT RESOLVED that the thanks of the Society be extended to the Chamber through Mr. Charles Genella, Convention Representative of the Chamber of Commerce.

C. The Board of Commissioners of the Port of New Orleans has kindly provided the ship "Good Neighbor" for tours of the Port of New Orleans by members of the Society and their wives. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to Mr. Edgar Bright, President, Board of Commissioners, and through him to the Board.

D. The assistance and cooperation of the members of the staff of the Jung Hotel contributed to the success of the Meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mr. Emmett J. Bieger, Director of Sales, Jung Hotel, and through him to other members of the staff of the Jung Hotel.

E. The interest and planning of the Committee for Ladies' Entertainment contributed greatly to the pleasure of the ladies attending the Meeting. THEREFORE BE IT RESOLVED that the Society expresses its thanks and appreciation to Mrs. Joseph S. D'Antoni, Chairman, and individually to the following Committee members: Mrs. Paul C. Beaver, Mrs. G. J. Buddingh, Mrs. John P. Fox, Mrs. William W. Frye, Mrs. H. E. Meleney, Mrs. Joseph H. Miller, Mrs. Robert L. Simmons, Mrs. Clyde Swartzwelder, and Mrs. Waldo L. Treuting.

F. The foresight and planning of the Local Committee on Arrangements were essential contributions to the success of the Meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Drs. Clyde Swartzwelder, Arthur J. Walker and Rodney C. Jung, Co-Chairmen, and Committee Members as follows: Miss Iris Krupp, Drs. Paul C. Beaver, Joseph S. D'Antoni, Edmond L. Faust, Ernest C. Faust, John P. Fox, William W. Frye, Henry M. Gelfand, Marion W. Hood, Maxwell E. Lapham, Henry E. Meleney, Albert Miller, Joseph H. Miller, Emma S. Moss, John H. Seabury, Robert L. Simmons, and Robert G. Yeager, Messrs. Thomas R. Read, Jr., and Kenneth M. Smoot.

G. The Hospitality Sessions and facilities at the Meeting were a most important contribution to the pleasure of the individual members and guests and to the success of the Meeting as a whole. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be extended to:

(1) Dr. Joseph S. D'Antoni, President, Standard Fruit and Steamship Company, and his company, for the Hospitality Session on November 1.

(2) Dr. Eugene H. Payne and Mr. M. B. Haggerty, Parke Davis Company, and their company, for the Hospitality Session on November 2.

(3) Mr. H. B. Fonda, Burroughs Wellcome and Company, and his company, for a contribution of $300 for Ladies' Entertainment and for other expenses of the Meeting.

(4) Mr. Kenneth M. Smoot, Winthrop Laboratories, and his company for 3 coffee sessions.

(5) Mr. J. Hugh Knight, A. S. Aloe Company, and his company, for a coffee session.

H. The Commercial Exhibits added materially to the interest of the Meeting. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Society be expressed to Mrs. Herma H. Hoefler of the American Foundation for Tropical Medicine, who served as Chairwoman of Commercial Exhibits Arrangements, and through her to the commercial exhibitors.

The Scientific Exhibits were of outstanding interest. THEREFORE BE IT RESOLVED that

the thanks and appreciation of the Society be expressed through the Louisiana State and Tulane University Schools of Medicine, the Army Medical Service and the Office of the Surgeon General, U. S. Army and the Pan American Sanitary Bureau to the individuals who so painstakingly developed these exhibits.

V. Editors of Society Publications.

A. Dr. Lewis W. Hackett, Editor of the American Journal of Tropical Medicine and Hygiene is to be commended for his efforts which have resulted in a Journal of outstanding quality. THEREFORE BE IT RESOLVED that an expression of thanks on behalf of the Society be extended to him.

B. Having ably edited the timely and interesting Tropical Medicine and Hygiene News for a period of years, Dr. Leon Jacobs resigned at the end of 1955 due to his acceptance of the post of Editor of the American Journal of Parasitology. He was ably succeeded by Dr. Louis J. Olivier. THEREFORE BE IT RESOLVED that an expression of appreciation of the Society be extended to them.

VI. Whereas, the number of mosquito species considered to be of public health importance in this country has increased greatly in recent years due to the detection of naturally acquired encephalitis virus in 13 species, the increasing importance of this infection in man, broadening public health standards and the increasing exposure of man to mosquitoes due to various socioeconomic trends,

And whereas, lack of knowledge of the biology and control of many species now recognized as having public health importance has been of increasing concern to public health and mosquito control agencies,

And whereas, since the last meeting of the Society, resolutions pertinent to these lacunae have been passed by the Association of State and Territorial Health Officers, the American Public Health Association and many other state and national mosquito control and public health associations,

THEREFORE BE IT RESOLVED that this Society go on record as supporting the need for more adequate work in this field by the U. S. Public Health Service in cooperation with state and territorial health departments and by academic institutions and granting agencies involved in this area of research, and that the Secretary of the Society be instructed to transmit advice of this action to appropriate parties.

Following the reading of the resolutions by J. M. Henderson, A. B. Sabin entered a resolution from the floor expressing the appreciation of the Society for the work of the Secretary-Treasurer during his four years in office.

These various resolutions were accepted by a voice vote, and a motion for adjournment was passed at 9:23 A.M.

Respectfully submitted for publication,
JOHN E. LARSH, JR.
Secretary-Treasurer

SCIENTIFIC AND SPECIAL SESSIONS

A total of seven half-day sessions was held, six regular sessions for the presentation of papers, and one special session for a symposium. Four of the regular sessions were presided over by the President, Asa C. Chandler, one (Session B, Thursday morning, November 1) was presided over by the President-Elect, Justin M. Andrews, and the Vice-President, Willard H. Wright, and one (Saturday morning, November 3) was presided over by the President-Elect, Justin M. Andrews. The symposium (Thursday afternoon, November 1) was moderated by Norman R. Stoll. In addition to these seven sessions, there was a dinner session (Friday, November 2). William W. Frye served as Toastmaster. N.

Paul Hudson, Chairman of the Award Committee, presented the Walter Reed Medal to Karl F. Meyer, and the President, Asa C. Chandler, presented an address on, "The Interrelations of Nutrition and Infectious Diseases in the Tropics."

2:00 P.M., October 31

*1. THE TWENTY-FIRST ANNUAL CHARLES FRANKLIN CRAIG LECTURE: *The Role of Pharmacology in Determining the Rational Use of Therapeutic Agents.* (30 mins.) LEON H. SCHMIDT, Cincinnati, Ohio. Introduced by G. ROBERT COATNEY, Chairman of the Lectureship Committee.

2. *The Pharmacological Basis for the Prolonged Protective Activity of Pyrimethamine.* CARL C. SCHMIDT and JEAN IHPIG (Introduced by L. H. SCHMIDT), Christ Hospital Institute of Medical Research, Cincinnati, Ohio.

Studies in human and simian malarias (*Plasmodium vivax* and *P. cynomolgi*) have shown that single doses of pyrimethamine confer protection against trophozoite induced infections for protracted periods. This protection has been attributed (Goodwin) to the presence of a metabolite of pyrimethamine which persists long after the parent drug has been excreted; this metabolite was characterized by high activity as a folic acid antagonist (in the *Streptococcus fecalis* system). The present report, dealing with a systematic study of the metabolic fate of pyrimethamine in man and rhesus monkey, was designed to test the above hypothesis. Both chemical and microbiological methods (*S. fecalis*) were used in assaying blood serum and urine for pyrimethamine content. Chemical assays showed that sera of both humans and monkeys contained measurable amounts of pyrimethamine for at least six days after dosage and probably longer. In the six day period, the human subjects excreted approximately twelve per cent of the ingested dose, the monkeys from twenty to forty per cent. Isolated examinations indicated continued urinary excretion of pyrimethamine by human subjects for at least fourteen days. Throughout the studies on sera and urine, chemically determinable pyrimethamine equaled or exceeded that which could be measured microbiologically. These findings offer no support for Goodwin's hypothesis, but rather suggest that tissue retention of pyrimethamine is considerable and in itself is adequate to account for the prolonged effectiveness of this drug against infections with *P. vivax* and *P. cynomolgi* in man and rhesus monkey.

3. *Paper Electrophoretic Studies on the Proteins of Avian Malarial Serum.* LEWIS A. SCHINAZI, University of California at Los Angeles, Los Angeles, Calif.

The serum proteins of pigeons have been partitioned by zone electrophoresis on filter paper, and studied with respect to the quantitative and qualitative changes occurring as a result of infection with strain 1Pl-1 of *Plasmodium relictum*. A modification of the horizontal paper-strip apparatus of Grassman and Hannig was employed. 0.04 ml samples of serum were applied to Whatman #1 filter paper strips (35 x 3.75 cm), and run in a veronal-acetate buffer of pH 8.6 and ionic strength 0.1, at a constant current of 0.4 ma. per cm width, for a period of 19 hours at 23–25°C. The oven-dried strips were stained with Brom Phenol Blue for proteins, and with Oil Red O for lipids. Analyses were performed in the Spinco Analytrol, an automatic recording and integrating densitometer.

The average total serum protein level, as measured colorimetrically by the biuret reaction, fell from a normal value of 2.6 g per 100 ml to 1.8 g. The alpha, beta and gamma globulins showed average increases of 2.0, 7.3, and 10.0%, respectively, during the course of the infection, and the albumin declined 40% of normal.

The most significant feature of the study was the clear partition of a component with mobility greater than that of albumin, heretofore undescribed for malarial serum. This unusual fraction made its initial appearance during the post-crisis phase of the infection, rose gradually to a concentration of 30% of

* To be published in the JOURNAL.

the total proteins present, and then disappeared abruptly from the serum. Preliminary studies on the characteristics of the fast component indicate that it contains nucleic acids and lipids in addition to protein. Marked fluorescence was also observed after exposure to ultra-violet light.

It is believed that this pre-albumin serum fraction arises as an indirect consequence of stimulated reticulo-endothelial activity, rather than as a direct result of the hemolytic anemia produced by the infection. Correlative studies on the histopathology of the liver, spleen and bone marrow are currently being pursued, with a view toward further clarification of the nature, source and possible role of the fast-moving component in the disturbed metabolism of the malarial animal.

4. *Further Studies on the* in vitro *Cultivation of the Mosquito Phase of* Plasmodium relictum. GORDON H. BALL and JOWETT CHAO, University of California, Los Angeles, Calif.

Oocysts of *Plasmodium relictum* (canary strain 1Pl-1) attached to the stomach wall of *Culex tarsalis* and *C. stigmatosoma* have been cultured previously *in vitro* in a complex "synthetic" medium. They were observed to undergo a threefold increase in size in 4 or 5 days. The rate of development was below that of *in vivo* controls and full maturity was never obtained, although the gut tissue of the host continued contraction for as long as 5 weeks.

As a new approach to the problem, oocysts of about 25u in diameter were sterilely dissected from the stomach wall of *C. tarsalis* and cultured in hanging drops and in Carrel flasks, at constant temperature of 21°C. The Carrel flasks were either kept stationary or rocked and were either sealed or aerated with a sterile mixture of 5% carbon dioxide and air.

The medium used was composed of groups of ingredients consisting of a balanced salt solution, phosphate and carbonate buffers, dextrose, amino acids, water soluble vitamins, purines and pyrimidines, chick serum, and chick embryo extract. Variations in the concentrations and combinations of the above ingredients were tried, with a view toward simplifying the medium. However, to date, no great simplification has been found favorable for the development of the oocysts.

In both hanging drops and Carrel flasks, oocysts were transferred every other day. Some of them were maintained as long as 3 or 4 weeks, with increases in size and changes in internal structure, but none was brought to maturity.

Currently, attempts are also being made to cultivate the pre-oocystic stages of the parasite, using blood drawn from mosquitos which have bitten infected canaries.

This work was aided by grant No. 1857 from the U.S. Public Health Service.

†5. *An Evaluation of the Intravenous Use of Anti-malarial Drugs with Reports of 87 Intravenous Injections of Quinacrine and 76 of Chloroquine Solution.* A. J. WALKER and L. H. DE GENERES, Tulane University of Louisiana, School of Medicine and Charity Hospital of Louisiana, New Orleans, La.

As each year of successful malaria control by residual insecticides passes, the greater becomes the possibility of sudden outbreaks of malaria. People become virtually non-immune after three years of freedom from the disease. Epidemics comparable to that of Ceylon in 1935 could occur in several parts of the world. Adequate stocks of camoquin, chloroquine and atabrine and primaquine should be stock-piled at several points in those areas of previously high endemicity now. If these are never used the initial cost would be compared to a very low life insurance premium. Any serious interruption of the current world-wide programs because of political or economic disturbances would be disastrous.

Blackwater fever and malaria cases with very high parasitemias are virtually unknown today. Should malaria return a great many of the new cases will be acute. It therefore seems appropriate at this time to outline some of the facts about parenteral medication for the younger physicians who have perhaps never seen malaria.

The wide-spread opposition to the use of intravenous medication found in the literature is apparently derived from much animal experimentation but in the case of

† Read by W. A. Sodeman.

atabrine on only 60 injections 0.05–0.5 gm given to 21 persons.

The present paper reports 86 doses of 0.2 gm atabrine powder in 10 cc. of distilled water given intravenously; also 75 intravenous injections of 5% chloroquine solution; all without reaction as the injections were *given very slowly*.

6. *The Morphology of* Toxoplasma gondii *as Revealed by Silver Impregnation Studies.* MORRIS GOLDMAN, RUSSELL K. CARVER, and ALEXANDER J. SULZER, Communicable Disease Center, Chamblee, Ga.

Fluorescent antibody studies with toxoplasma revealed the presence of a long, thread-like appendage on the organisms following exposure to 0.1 per cent formalin in saline. Attempts to demonstrate this structure with silver impregnation methods have not, as yet, been successful, but a variety of other structures, some of which have not been described before, were clearly demonstrated. These structures and what appears to be a method of division not previously described for toxoplasma will be demonstrated with projection slides of photomicrographs.

7. *A Hemagglutination Test for Toxoplasmosis.* LEON JACOBS and MILFORD N. LUNDE, National Institutes of Health, Bethesda, Md.

The *in vitro* dye test for toxoplasmosis is the most useful serological procedure now available for diagnosis. However, it has the considerable disadvantage that it requires maintenance of the parasite in the laboratory, and the routine handling of large numbers of infectious organisms. It is therefore not a practicable procedure for routine hospital use.

In the hope of developing a practical routine test, antigens prepared in various ways from toxoplasmas derived from the peritoneal exudates of infected mice have been tested in a hemagglutination test. The antigens are bound to washed tannic-acid-treated red cells according to the methods outlined by Stavitsky (1954). Treated red cells at an optimal concentration are then incubated with standard dilutions of test serums and controls.

Unwashed, frozen and thawed, peritoneal exudates of infected mice failed to give satisfactory results. Also unsatisfactory was an acid-soluble protein fraction obtained from washed and lysed parasites. However, parasites washed out of exudate and then lysed in distilled water, produced useful antigens. Preliminary tests with these antigens have furnished information on optimal concentrations, and have yielded results on coded sera which compare favorably with the dye test titers.

8. *Variations in the Results of the Methylene Blue Dye Test for Toxoplasmosis Observed During a Four Year Period.* ALEXANDER J. SULZER, M. M. BROOKE, and RUTH H. GOGEL, Communicable Disease Center, Atlanta, Ga.

The methylene blue dye test (Sabin and Feldman, 1948) has been performed routinely for four years by this laboratory for the serologic diagnosis of congenital toxoplasmosis. Although it has been of value in these cases, considerable day-to-day variation in the reaction has occurred as determined by the set of controls included in each test. Using standard procedures, the yield of acceptable tests for diagnostic purposes has ranged between 10 and 65 per cent of those performed during given periods.

Careful attention to factors like cleanliness of glassware, suitability of accessory factor serum, methods of maintaining infections of *Toxoplasma gondii* in mice, age and weight of mice used, and other technical details has not made it possible to obtain consistently satisfactory tests.

As reported by others, the observations indicate that the antigenic property of the peritoneal fluid of infected mice used in the test is responsible for variations in the reactions. Experiences are reported on: (a) the results obtained by using different lines of the RH strain procured from other laboratories, (b) the results obtained by using organisms derived from individual mice infected with the same line of toxoplasma, and (c) attempts to improve the antigen by washing organisms free of the liquid portion of the peritoneal fluid.

9. *An Evaluation of the Specificity of the Fluorescence Inhibition Test for Toxoplasma Antibodies.* MORRIS GOLDMAN and RUSSELL K. CARVER, Communicable Disease Center, Chamblee, Ga.

The fluorescence inhibition test is based

upon the fact that antiserum can inhibit the specific staining of toxoplasma organisms by fluorescein-labelled antibody. The specificity of the results obtained in testing sera by this method has been studied by two methods: 1) The relationship between methylene blue dye test results, clinical information and the results of the inhibition test on selected human sera; and 2) A comparison of inhibition test results on sera from animals before and after immunization with a variety of antigens other than toxoplasma. In the first category, results obtained with 218 sera indicate that the fluorescence inhibition test and the dye test are entirely comparable in their demonstration of positive and negative sera. In the second category, sera from 100 different animals immunized with 26 different antigens have shown no differences in reaction before and after their course of immunization, although they generally developed high titers to the specific antigen with which they were immunized.

*10. *Preliminary Studies on Toxoplasma Antibodies in Guatemala and Costa Rica.* COLVIN L. GIBSON and NELL COLEMAN, National Institutes of Health, Memphis, Tenn.

*11. *Toxoplasma Infections in Animals Associated with a Case of Human Congenital Toxoplasmosis.* COLVIN L. GIBSON and DON E. EYLES, National Institutes of Health, Memphis, Tenn.

*12. *Toxoplasmosis in the Domestic Cat.* FRANCIS E. JONES, DON E. EYLES, and COLVIN L. GIBSON, National Institutes of Health, Memphis, Tenn.

* Abstract withheld from published group upon request of author(s).

13. *Isolation of Toxoplasma at Autopsy from a Patient with Chorioretinitis.* DON E. EYLES and WHEELAN D. SUTLIFF, National Institutes of Health and Kennedy Veterans Hospital, Memphis, Tenn.

TG, a 62 year old white male carpenter came to autopsy May 14, 1956, and the clinical diagnosis of carcinoma of the lung metastatic to the brain and adrenal glands was confirmed as the primary cause of death.

This patient had had a number of previous admissions to Kennedy Veterans Hospital for illnesses unrelated to the terminal illness. In 1951 he gave a history of decreased visual acuity over a period of 12 years, and examination at this time disclosed an extensive, healed chorioretinitis of the left eye. Subsequent examinations confirmed the original findings and on Jan. 9, 1956 serum was taken for dye testing as a part of a study of the etiology of eye infections. The end-point titer was found to be 1:256.

At autopsy, brain tissue from several loci was pooled, then ground in a Waring blendor and injected into uninfected white mice. The diseased eye was also taken and a small portion ground and put into mice, the majority being retained for histologic study. On the fourth passage in mice, *Toxoplasma* organisms were detected in small numbers in two mice. Subsequent passages are being made. No *Toxoplasma* have been recovered from the eye through three mouse passages, and histologic study is not yet complete.

9:00 A.M., November 1

(Scientific Session A)

14. *The Imported Fire Ant,* Solenopsis saevissima *Var.* richteri *as an Agent of Disease.* RODNEY C. JUNG and VINCENT J. DERBES, Tulane University School of Medicine, New Orleans, La.

The imported fire ant, *Solenopsis saevissima* var. *richteri* is a tropical agent of human disease which has become well established in the United States. Its range in this country is extending and its incidence increasing. This insect, which was introduced into Mobile before 1930, now infests at least 10 southern states and has become a serious economic problem destroying crops and quail in that region. It is of interest medically because of the ferocity with which it attacks human beings. The stinging apparatus is similar anatomically to that of the honey-bee but the poison differs in that it is a powerful necrotizing agent. The lesion produced characteristically goes through the stages of wheal,

vesicle and pustule. Pustulation is not evidence of secondary infection since the pustules are usually sterile. Healing of the lesion is accompanied by cicatrization. Many thousands of persons are attacked by these insects yearly and most of them endure the discomfort of the stings without consulting a physician. However sometimes large hemorrhagic bullae, infectious ezematoid dermatitis or massive swelling may be produced, which require medical attention. Some individuals may develop other types of allergic reactions including fatal anaphylaxis. Antibiotics, antihistamines systemically and topically and topical hydrocortone have been found to be valueless in treatment.

†15. *Salivary Gland Chromosomes in* Anopheles quadrimaculatus. GORDON B. WOLCOTT, National Institutes of Health, Columbia, S. C.

The salivary gland chromosomes of mosquito larvae are similar in structure to those of other larval Diptera, being polytene and very large. These chromosomes have been studied in an inbred laboratory strain of *A. quadrimaculatus* and in larvae obtained from wild-caught *A. quadrimaculatus*. The banding pattern has been partially determined and the sex chromosome identified. Inversions and duplications were found in both strains. There are certain differences between the wild strain and the laboratory strain.

16. *Use of Insecticide-Impregnated Cord for House Fly Control.* JOHN W. KILPATRICK and H. F. SCHOOF, Communicable Disease Center, Savannah, Ga.

In previous studies, cotton cords 3/16″ and 3/32″ diameter impregnated with a 10 percent parathion-xylene solution, and limited tests with 3/16″ diameter cords impregnated with a 25 percent Diazinon-xylene solution, have provided excellent to satisfactory house fly control in dairy barns, chicken ranches, rural areas, and military dining halls for an entire fly season in southeastern Georgia.

Studies in 1956 were designed to further evaluate the efficiency of the cord technique for fly control in dairies, chicken ranches and rural areas with Diazinon.

† Read by title.

17. *Toxicological Studies on Some Organic Phosphorus Insecticides of Public Health Importance.* THOMAS B. GAINES, WILLIAM F. DURHAM, WAYLAND J. HAYES, JR., and KENNETH D. QUARTERMAN, Communicable Disease Center, Savannah, Ga.

Investigations on the mammalian toxicology of some organic phosphorus insecticides of public health importance are presented. Among the compounds considered are chlorthion, malathion, diazinon, EPN, parathion, and DDVP. The data include acute dermal and oral LD_{50} values, studies of respiratory toxicity, effect on rats of various levels of toxicant incorporated into their diet, and results of screening tests for possible paralytic effect. A discussion of the relative toxicological hazard involved in the use of these various compounds is given.

18. *A Mechanically Produced Insecticidal Aerosol from a Pocket Size Instrument.* W. E. DOVE, Millersville, Md.

Liquefied-gas aerosols afford a convenient means for disinsectizing buildings and the commercially made bombs are readily available in this country. In some foreign countries, liquefied gas is not always available, and at times it is difficult for one to obtain one of these bombs. For such purposes and for reasons of economy, there is a need for an instrument that can mechanically disperse aerosol particles from a concentrated insecticide.

In 1945, Lindquist, Schroeder and Knipling showed that a 10% solution of an insect toxicant properly dispersed in the air by mechanical means was essentially as effective as a 1% solution applied with a conventional sprayer. In 1946, Schroeder and Lindquist reported upon four models of aerosol sprayers. These devices made use of cylindrical tanks with a leather compression cup fitted on one end, in such a manner that compressed air was forced over the orifice of a siphon tube at the forward end of the tank.

In extending this development, some improvements in design by entomologist, Dr. Wm. Dickison, and his engineering associates have resulted in an efficient and leakproof instrument. It has sufficient capacity to last for more than an entire season for

ordinary household use. The performance, the construction, the limitations and the use of a suitable spray concentrate for control of resistant flies and mosquitoes are discussed in this paper.

19. *A Practical Philosophy of Vector Control in the United States.* JOHN M. HENDERSON, Communicable Disease Center, Savannah, Ga.

In areas where arthropod-borne diseases are ranking causes of death and disability, control programs which are specifically limited to the disease vector are often indicated. In the United States, vector-borne diseases are no longer primary public health problems but there are still many situations requiring control of non-agricultural arthropods for the betterment of mankind.

In addition to the control of classical vectors of disease, in recent years it has become a recognized public health function to control certain species of mosquitoes which are not responsible for the current transmission of communicable disease, on grounds of interference with mental equanimity (i.e. mental health) and positive health, and potential vectorial capacity.

Quite apart from this, under prevailing conditions in the United States it is often unrealistic to attempt to separate the control of classical arthropod vectors from the control of related species which are detrimental to man in other respects.

In California, for example, mosquito-borne encephalitis is an important public health problem, the vector of which at the present time can be effectively controlled only in the immature stages. This vector commonly produces in association with other mosquito species of high pestiferous importance. About ninety percent of the nearly 4 million dollars being spent each year in California are local funds. The dominant motivating interest of the public in supporting this expenditure is to secure relief from acute and widespread discomfort. The activities which are financed by these funds result in the joint control of classic vectorial and pestiferous mosquito species. Research results on improved methods of pest mosquito control, at times can be more helpful in improving the adequacy of vector control than a more direct line of approach.

Efforts to control only the classic vector would variously be operationally impractical, result in loss of local funds and local organized mosquito control, and in the favoring of expedient control measures over more comprehensive preventive and eliminative measures.

From an epidemiologic standpoint, an adequate understanding of the ecology of control is necessary in this instance rather than the case-finding viewpoint.

20. *A Method for Securing Bacteriologically Sterile Snails* (Australorbis glabratus). ELI CHERNIN, Harvard School of Public Health, Boston, Mass.

Snails maintained in a sterile environment would be useful tools for studies on molluscan nutrition, physiology, and host-parasite relationships, and would also be of particular value in research on the control of snail vectors by biological means.

Since the bacteriological sterilization of adult aquatic snails is manifestly difficult, efforts were directed toward a means of surface-disinfection of the egg masses of *Australorbis glabratus*, with subsequent hatching of snails under sterile conditions. Such a technic was developed but the results of sterility tests were inconsistent. Further study indicated that in some cases contaminants were within and not on the surface of the eggs and thus escaped disinfection. Consequently, eggs were dissected from the egg mass, treated, and then isolated in separate tubes for hatching.

Technic: Egg masses of laboratory-reared *A. glabratus* were removed from vegetation and kept until mature. Carefully selected masses were then placed in a weak solution of detergent, and, under a low-power microscope, individual eggs containing fully mature embryos were dissected free. The eggs were then briefly exposed to a weak solution of sodium hypochlorite which served as the disinfecting agent. This solution was then removed, the eggs were washed and then placed in individual tubes containing a small volume of a suitable salt solution plus penicillin and streptomycin. Treated snails were maintained at 28°C.

With the concentrations of NaOCl employed and a suitable treatment interval, 75–100% of the treated eggs hatch within a few days. Sterility tests in fluid thioglycol-

late and in several other bacteriologic media have been negative in 75–100% of trials.

Studies on the cultivation of *A. glabratus* hatched under these conditions are currently in progress.

21. *Relationship of Temperature to Molluscocidal Activity.* ROBERT E. KUNTZ, Naval Medical School, NNMC, Bethesda, Md.

Molluscocidal efficacy and potential have been described in terms of chemical structures, effects of selected compounds upon the metabolism of the molluscan hosts, or erratic and varying results in evaluating promising chemicals have been attributed to characteristics of the different types of waters and habitats in which applied. Although it is assumed that the use of chemicals is the logical approach to mollusc control, suggestions have been made for biological control by the introduction of predators or infectious agents into the snails' habitat. Regardless of the approach, snail control must be accomplished on an economically sound basis employing the attributes of nature when feasible.

Although temperature is accepted as an important factor in regulating natural, physiologic and chemical processes, little thought has been given to temperature in terms of practical or economic snail control. A study of the efficacy of several molluscocides against the vectors of schistosomes in Egypt over a period of 2½ years has demonstrated clearly the relationship of experimentally controlled and seasonal temperatures upon molluscocidal activity. The marked increase in molluscocidal activity of chemicals tested at elevated temperatures has indicated that application of molluscocides, when feasible, should be planned for the warmer months of the year.

22. *Parasites of Rats Collected in the City of Detroit.* DOMINIC L. DEGIUSTI, Wayne University, Detroit, Mich.

226 *Rattus rattus norvegicus* collected from 126 different locations in the city of Detroit have been examined by careful searching through the organs and tissues for helminth parasites. The following parasites were found: *Heterakis spumosa* 77%, *Capillaria gastrica* 52%, *Trichosomoides crassicauda* 34%, *Nippostrongylus muris* 26%, *Hymenolepis diminuta* 10%, *Hymenolepis nana* 13%, *Cysticercus fasciolaris* 25%, *Fibricola cratera* 0.5%.

150 live trapped rats were examined for ectoparasites, by wet-combing the animals in 70% alcohol and collecting the arthropods on to filter paper by filtration of the alcohol. Previous trials demonstrated dry combing to be unreliable and that dead trapped or gassed animals also resulted in unreliable incidences. Arthropod incidences in Detroit rats are as follows: *Xenopyysila cheopis* 23.7%, *Nosopsyllus fasciatus* 27%, *Ctenocephalides canis* .60%, *Polyplax spinulosa* 80.5%, *Bdellonyssus bacoti* 15.8%, *Echinolaelaps echidnius* 7.7%, *Radfordia sp.* .4%, *Notoedres notoedres* .6%, Mesostigmatid mites unidentified 1.9%. On the basis of actual numbers present *Xenopsylla cheopis* is the most abundant flea on Detroit city rats. As compared to other surveys, Detroit rats show a much greater incidence of *Radfordia*. This is believed to be due to the methods used for recovery in the Detroit survey.

1200 rat diaphragm, 200 dog diaphragm, and 23 cat diaphragm were examined for the presence of *Trichinella spiralis*. 2% of the rats, 3.9% of the dogs, 4.3% of the cats were found infected with *Trichinella spiralis*.

House address source of rats was recorded. No rats from slaughter house areas have been included in this survey of *Trichinella spiralis* incidence in rats.

*23. *Parasite Incidence in an Institutional Population During Two Years of Observation.* GEOFFREY M. JEFFERY, National Institutes of Health, Columbia, S. C.

*24. *The Suppressive and Prophylactic Action of Puromycin (Stylomycin) Against Intestinal Parasites.* MARTIN D. YOUNG, JOE E. FREED, and GEOFFREY M. JEFFERY, National Institues of Health and South Carolina State Hospital, Columbia, S. C.

†25. *The Possible Influence of pH and Specific Gravity on the Formalin Ether (406th MGL) Technic in the Concentration of Fecal Eggs and Cysts of Certain Parasites.* L. S. RITCHIE, S. LIN, L. P.

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* Abstract withheld from published group upon request of author(s).

† Read by title.

FRICK, A. P. MOON, J. E. WILLIAMS, S. ASAKURA, and Y. HISHINUMA, 406th Medical General Laboratory, San Francisco, Calif.

Formalin-fixed feces was prepared in aqueous solutions with pH values of pH 4, 7 and 10 (buffered) and concentrated in parallel by means of the formalin ether technic. Eggs of *Ascaris* and *Schistosoma japonicum* were recovered best at pH 10. At pH 4 *Ascaris* egg recovery was reduced enough to affect diagnostic efficiency, while for *S. japonicum* the reduction was excessive. Conversely, hookworm eggs were recovered effectively at pH 4 and 7, but almost none were obtained at pH 10. Although pH 7 was optimal for whipworm, the difference was not sufficient to influence diagnostic efficiency. For protozoan cysts, the pH value of 7 was the one of choice; however, the difference was minimal.

Alcohol was added to each of the three buffered pH solutions to the extent of 20 per cent, as a means of determining if lowered specific gravity would affect the results. A marked increase of whipworm, *Ascaris* and *S. japonicum* eggs was noted, particularly for pH 7. Corresponding influence on hookworm was not evident, and a slight loss of efficiency on protozoan cysts was apparent.

Most impressive was the increased diagnostic benefit obtained for *S. japonicum*. When pH was the only factor involved, the value of 10 was optimal, but in combination with alcohol best results were obtained at pH 7. With this combination of factors, it appears that a level of efficiency for recovery of *S. japonicum* can be attained comparable to that afforded by the MIF (merthiolate-iodine-formalin) concentration procedure.

‡26. *Some African Tropical Diseases*. EDWARD K. MARKELL, University of California Medical School, Los Angeles, Calif.

Motion picture, 16mm., color, sound, 15 minutes. Presents hospitals and research centers in Cairo, Nairobi, Kampala (Uganda), Brazzaville, and Harbel (Liberia), and some interesting clinical cases encountered at each institution. The diseases shown include schistosomiasis mansoni, kala-azar, leprosy, dracontiasis, kwashiorkor and African sleeping sickness.

‡ Film.

26a. *Species Crossing in* Oncomelania. E. D. WAGNER, D. INABA, and L. W. WONG, School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, Calif.

Oncomelania nosophora was crossed with *O. quadrasi* and with *O. formosana*. *O. quadrasi* was crossed with *O. formosana*, thus successfully completing all possible crossings with the three species studied. F_2 offspring were produced in the first 2 species listed and F_1 offspring in the others. Matings to obtain F_2 in all are in progress. In each case crossings were made using combinations of male and female of each species, so that a male *O. nosophora* and a female *O. quadrasi* were mated and the reverse, a female *O. nosophora* and a male *O. quadrasi*, etc. The fourth species, *O. hupensis*, is being included in the study at the present time.

The findings indicate that these snails are not biologically (reproductively) distinct species.

The significance of the findings should be examined in the light of the failure of the "geographical strains" of the parasite, *Schistosoma japonicum*, to develop in all of the oncomelanid "species."

26b. *Infection of Chick Embryos by Crithidia from the Phytophagous Bug*, Euryophthalmus davisi. R. B. MCGHEE, University of Georgia, Athens, Ga.

One hundred per cent of the bugs *Euryophthalmus davisi* was found to be infected with a crithidia (possibly *Crithidia euryopthalmi* (McCulloch, 1917)). The flagellate was successfully cultured on N.N.N. medium. After several passages the protozoan was introduced to a medium similar to N.N.N. but with duck blood substituted for rabbit blood. A heavy growth ensued. At the time of maximum growth, the culture was diluted with saline and placed on the chorioallantoic membranes of developing chick embryos. The temperature of incubation was reduced from 37° to 30°C. Four days later it was possible to demonstrate considerable multiplication of the crithidia (now possessing a leptomonad structure). Parasites were free and in macrophages of the embryo. The protozoa have survived and multiplied for three successive passages.

Cultures from the chorio-allantoic fluid and from the liver have demonstrated the presence of parasites in these tissues. *Crithidia* are now being maintained in embryos incubated at a temperature of 37°C.

9:00 A.M., November 1

(Scientific Session B)

27. *Tularemia in Texas.* J. S. WISEMAN, R. B. EADS, and J. V. IRONS, Texas State Department of Health, Austin, Texas.

Five hundred and fifty-five cases of tularemia have been reported in Texas by physicians during the 10-year period 1946–1955. In 1950 the practice of sending a questionnaire to the reporting physicians relative to each case was initiated. During 1950–1954 288 cases were reported with histories being obtained for 126 cases, 110 of which were laboratory confirmed. Sixty-six (52.4%) gave a history of tick bite, 41 (32.5%) a history of rabbit, rodent or other animal contact, 5 (4%) a history of both tick and animal contact and 14 (11.1%) were uncertain of animal or tick contact.

One hundred and four (82.5%) of the 126 cases were reported from 35 counties in the eastern one-fourth of the state with the preponderance of cases coinciding with the April, May and June peak of greatest tick abundance. This section of the state is well within the range of the common eastern cottontail rabbit, *Sylvilagus floridanus*. The predominant species of tick is *Amblyomma americanum*, the "lone star" or "speck-backed" tick. It occurs in tremendous seasonal concentrations in the field and readily attacks man and other animals.

The fact that tularemia in Texas essentially is limited to the eastern part is emphasized by the numerous wild and domestic animal sera which have been tested and the thousands of ticks and other arthropods inoculated into laboratory animals in studies conducted in other parts of the state with negative results. The projects have included a typhus study in South Texas, a Q fever investigation in South Texas, plague studies in West Texas and a "Bullis fever" investigation in Central Texas.

Current attempts to isolate tularemia organisms from ticks and animals in East Texas are reported on.

*28. *The Prevalence of Dermal Sensitivity to Brucella Infection Among Patients with Cardiovascular Disease.* CHARLES M. CARPENTER, JOSEPH A. SCHWARTZ, and DONALD W. LEIK, School of Medicine, University of California, Los Angeles, Calif. and Medical Service, Veterans Administration Hospital, Long Beach, Calif.

Previous studies with experimental brucellosis in guinea pigs has demonstrated that *Brucella suis* not infrequently causes cardiovascular lesions in the myocardium of guinea pigs resembling those pathognomonic of rheumatic fever. As a result of this observation, an epidemiologic study was undertaken to determine if chronic or latent brucellosis bears any relationship to cardiovascular disease in man. Intradermal tests with Brucellergen were made to compare dermal sensitivity among hospitalized male patients with various types of chronic illness. The results revealed that approximately 60 percent of such patients with cardiovascular disease reacted positively to the test, whereas only 30 percent of the patients in the same age groups hospitalized for gastro-enteric disease exhibited evidence of dermal sensitivity to Brucellergen. The data suggest that chronic or latent brucellosis may have some etiologic significance in certain types of cardiovascular disease.

29. *An Analysis of the Most Recently Available Information on Etiologic Agents Responsible for Infant Diarrhea in the American Tropics and Subtropics.* VIOLA MAE YOUNG, Walter Reed Army Institute of Research, Washington, D. C.

The primary cause of infant mortality in most tropical and subtropical countries is infectious gastroenteritis, a disease which can be largely eliminated under improved hygienic conditions. Each of the etiologic agents presents an epidemiological problem

* Read by J. F. Kessel.

peculiar to itself, yet each has certain aspects in common with the others.

A direct correlation exists between strains isolated from man and those recovered from animals in the various areas reported. As would be anticipated, this holds true for infants as well as adults. Infection is possible any time after birth or, as the recovery of Salmonellae strains from placenta reveals, during birth.

Shigellosis is common in infants, though encountered less frequently in those under six months of age. Traditionally a finger and fly borne disease, there are several unexplained factors in the picture it presents in tropical areas.

The pathogenic *Escherichia coli*, originally described only from outbreaks of dysentery in hospital nurseries, are now believed to play an important role in gastroenteritis of the very young infant. From the available statistics it is apparent that these organisms can be frequently isolated from infants with diarrhea in some sections of the American tropics and subtropics.

It can be anticipated that the next few years will bring added information concerning the role of viruses in diarrheal disease. Coxsackie viruses have been isolated in considerable number from outbreaks of gastroenteritis in Brazil, but few virus studies have been made in regions other than Brazil and Mexico.

Further studies, encompassing bacterial, viral, and protozoan investigations of the same specimen, are essential to an accurate evaluation.

30. *Bacteriological Observations on Fevers of Undetermined Origin in Egyptian Village Children.* THOMAS M. FLOYD, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Short term fevers of obscure origin are common among children living under the compromised sanitation conditions of Egyptian villages. While a wide variety of bacterial, viral and parasitic diseases long have been known to be highly endemic in this environment, extensive etiological studies of these fevers have been hindered by limited laboratory facilities. Between 1952 and 1954, during the course of a general survey for virus infections in Egypt by the Virology Department of NAMRU-3, there was an opportunity to culture blood from a relatively large number of febrile children. By interest and necessity, search for bacterial agents was limited to members of the family *Enterobacteriaceae*.

Blood was obtained from 2,484 children, mostly between one and five years old, suffering from undiagnosed febrile attacks of not more than five days duration. Clots were cultured in enrichment broths and organisms identified by conventional biochemical and serological procedures.

Enterobacteriaceae were recovered from 125 (5%) of the specimens. Salmonellas were isolated from 99 (3.9%). *Salmonella typhi* was recovered from 65 of the children, *Salmonella paratyphia A* from 2, *Salmonella paratyphi B* from 7. *Salmonella paratyphi C* from 12 and *Salmonella typhi-murium* from 13. *Proteus morgani*, *Escherichia coli*, *Alkaligenes fecalis*, *Pseudomonas aeruginosa* and paracolons were found in the other 26 (1%).

Follow-up studies by local health officers failed to reveal a single instance in which these infections developed into typical cases of typhoid-paratyphoid fever. While it frequently has been observed that characteristic symptoms of typhoid may not appear in children, these data still would appear to lend evidence to the impression that, under highly endemic circumstances, conditioning by previous exposure may result in mild or asymptomatic infections.

31. *Prevalence of Syphilis in Burma, Analysis of Serologic Data Accumulated During the Period 1951–1952.* HAROLD A. TUCKER The Upjohn Company, Kalamazoo, Mich.

In 1951–1952 the W. H. O. venereal disease control team in Burma carried out a serologic survey in accessible areas in an effort to estimate realistically the prevalence and distribution of syphilis. V.D.R.L. (cardiolipin) qualitative tests were done on 11,827 specimens of serum taken in various parts of the country with an over-all prevalence of seropositivity of 16.6 per cent. On the basis of this and similar data from another source, and taking into account the likely contributions of yaws, malaria and leprosy to this figure, it was concluded that a fair estimate of the prevalence of syphilis would be in the range of 10 to 20 per cent. It was

considered remarkable that, within the limits of the data, this prevalence seemed to apply not only in large riverine cities such as Rangoon and Mandalay, but in the smaller towns and possibly even in some rural areas as well.

32. *Effects of Flotation on the Immunogenic Capacity of the Influenza A Virus (PR8 Strain).* S. H. HOPPER and NEWTON KHOOBYARIAN, Indiana University School of Medicine, Indianapolis, Ind.

It was previously reported that the "Flotation Method" could be used for recovery of virus from water. At the same time the experiments indicated that there was an increase in the hemagglutinating activity of influenza A virus (PR8 strain) with a concurrent decrease in infectivity. This report represents an extension of the previous work and shows somewhat different results if phosphate buffer is used as a diluent for the virus instead of distilled water. There is the same decrease in infectivity but there is also a decrease in hemagglutinin titer instead of the previously observed increase when the virus was suspended in saline. In the light of these findings it was interesting to determine whether the antigenic characteristic of the virus had also been altered. Using the *in vitro* hemagglutination-inhibition procedure as well as *in ovo* neutralization tests a clearly demonstrable relationship is evident between the pre-aerated and post-aerated virus. Although the hemagglutinating and infective capacity of the virus was reduced following aeration in phosphate buffer, the virus retained its original antigenicity.

33. *The Enteric Viral Flora of a Population of Normal Children in Southern Louisiana.* HENRY M. GELFAND, JOHN P. FOX, and DOROTHY R. LEBLANC, Tulane University Medical School, New Orleans, La.

The natural transmission of poliovirus is being studied in a selected, representative population in southern Louisiana, utilizing routine monthly blood and stool specimens from an average of approximately 150 young "index" children from almost as many families. As a by-product of this study, a large number of viral agents has been isolated from fecal samples by means of tissue cultures of monkey kidney cell epithelium. Polioviruses of all three types, as well as many cytopathic agents not yet completely identified have been recovered. This paper presents data showing the distribution of positive isolations over a 24-month period, and the effect of such social variables as race and economic status and family size on the percentage positive.

From 3667 specimens, a total of 499 strains were recovered, of which only 170 were polioviruses. In both years there was a marked seasonal variation in the rate of virus isolation, the most important months being June through October, with a peak of 30% positive in July 1954. In both years, the highest percentage recovery was in the negro group, followed by the white-lower economic group, with the white-upper economic group producing the least number of strains. The influence of family size was not consistent. In 1954, large-family children excreted enteric viruses more frequently than did small-family children, but in 1955 this difference had disappeared.

No serious illness could be associated with these infections.

34. *Status of Yellow Fever in Middle America.* J. AUSTIN KERR, Pan American Sanitary Bureau, Washington, D. C. (on loan from The Rockefeller Foundation, New York, N. Y.)

The march of the current epidemic of jungle fever from Panamá to Guatemala in the years 1948 to 1956 is summarized. The results of field and laboratory studies done in northern Honduras and eastern Guatemala are described. There seems to be no reason why the virus should not move forward through the tropical rain forests of northern Guatemala into the Mexican states of Chiapas, Tabasco and Veracruz. The field studies involved capturing rain forest mosquitoes in Guatemala and shipping them alive under refrigeration to Panamá by air, as well as collecting monkey blood specimens for serologic studies and liver specimens from dead monkeys for histopathological and virological study. Limited observations were made of the variations in prevalence and abundance of forest canopy mosquitoes.

Numerous isolations of yellow fever virus, and of at least one strain of another neuro-

tropic virus, were achieved by inoculating mice with ground-up mosquitoes.

Two different ecological pictures emerged regarding howler monkeys and *Haemagogus* mosquitoes. Where *H. spegazzinii* and *H. mesodentatus* were dominant the monkeys were practically exterminated in a few weeks, with the concurrent rapid disappearance of the virus, but where *H. equinus* was the only *Haemagogus* the monkey mortality was low, and the virus remained present in a small area for as long as twenty months.

In Central America *Aëdes aegypti* has been so well controlled as to be practically eradicated; the species is still present in Mexico.

In the southern USA the species is widespread, but the intensity of the infestation is not accurately known. Comprehensive surveys are needed as a prelude to the indicated eradication measures.

35. *Steatorrhea in Tropical Sprue and Its Relation to Certain Types of Diet.* CONRADO F. ASENJO, RAFAEL RODRIGUEZ-MOLINA, and MARTA CANCIO, School of Tropical Medicine, University of Puerto Rico and Veterans Administration, San Patricio Hospital, San Juan, P. R.

A screening diet palatable and balanced containing 40–95 grams fat, 85 grams protein and 200 grams carbohydrate (2,200 calories) was administered to documented sprue cases over a ten day period in order to determine the presence or absence of steatorrhea. Individuals who showed steatorrhea under this diet, that is when the 24-hour metabolic total fat output in the feces was found to be over 6 grams, were subsequently submitted to the following dietary trials: a low fat diet (under 10 gms.) containing about 44 grams wheat gluten (2,200 calories); followed by another diet, low in fat (under 10 gms.) and low in gluten (traces). Each diet was administered over a ten day period.

Results

1. Ten patients showed steatorrhea when screening diet was given. Twenty-four hours fecal fat ranged from 7 to 27 grams with an average of 16.4 grams. (Within the range of 10 to 160 gms. of fat intake per day, the total fat output in the feces in a healthy adult is considered to be 3 to 6 grams daily.)

2. Eleven patients did not show steatorrhea while under a low fat (under 10 gms.) containing about 44 grams wheat gluten. Total fat for the group ranged from 2 to 6 gms. with an average of 2.0 gms. per 24 hours.

3. Eleven patients did not have steatorrhea when they were given a low fat (under 10 gms.) and low gluten (traces) diet. The average daily fat output for the group was 1.59 gms. per 24 hours.

Frequently, when patients showed steatorrhea under screening diet, the stools were observed to be large, liquid, soft or semi-solid, light colored and of foul odor. When diet was low in fat and in gluten diarrhea improved in some cases and stools became harder and darker in color.

36. *Veno-occlusive Disease of the Liver and Indian Childhood Cirrhosis.* DERRICK B. JELLIFFE, GERRIT BRAS, and KANAI L. MUKHERJEE, All-India Institute of Hygiene and Public Health, Calcutta, India, University College of the West Indies, Mona, Jamaica, and Calcutta School of Tropical Medicine, Calcutta, India.

The world incidence of cirrhosis of the liver in children was reviewed, with particular reference to the extreme commonness of the condition in two widely separated areas, Jamaica and India, especially the eastern part of the subcontinent.

The significance of veno-occlusive disease of the liver (V.O.D.) in the pathogenesis of cirrhosis in Jamaican children was described, together with its clinical picture and pathology.

A series of cases of Indian childhood cirrhosis were investigated in Calcutta, West Bengal. Liver biopsies were performed where permitted and one autopsy was obtained. Clinical features, possible etiological factors, laboratory data and histological findings were presented and compared with accounts in the literature. The most significant finding was that definite V.O.D. was present in the autopsied case, while two liver biopsies were suggestive. On this basis, together with evidence from the comparatively few previous reports of post-mortems, it is considered that the process known as veno-occlusive disease of the liver is responsible for at least some cases of Indian childhood cirrhosis.

37. *Eye Infections in a Punjab Village.* CARL E. TAYLOR, PREM VIR GULATI, and JOSEPH HARINARAIN, Harvard School of Public Health, Boston, Mass. and Christian Medical College, Ludhiana, Punjab, India.

There are few epidemiological surveys of the distribution of hyperendemic trachoma and related infections in a population. Although most studies of eye infections have concentrated on clinical and laboratory features, the basic etiology has not yet really been defined.

In a Punjab village a control program was started in the schools. Approximately 90% of the 200 children had eye infections resembling clinical trachoma with secondary infections. With daily applications of antibiotic ointment, a dramatic drop in infection rates was observed, but when treatment was stopped, infections recurred.

More than 95% of the 1,300 people in the village were examined in two weeks of house to house visiting by two doctors. 67% had trachoma-like eye infections. Infection rates were 63% at 1 year of age and 76% at 4 years of age. After that, rates gradually fell to 50–60% except for a slight rise when children started to school and in old age. Women during the childbearing period had consistently higher rates than men.

High caste Hindus had an infection rate of 63%, Jat Sikhs who are hard working farmers had 67%, and low caste Chamars had 78% (latter difference highly significant). The Chamar community was divided in two areas with different levels of sanitation and housing. The group crowded into a cluster of houses near the center of the village had a rate of 84% while another group with more space on the edge of the village had 68% infection (highly significant difference).

These findings suggest two conclusions:

(1) Trachoma transmission in the Punjab is primarily a mother and child infection with school infections being less important than has been reported from other areas. School treatment programs will probably not have maximum effectiveness.

(2) The relation of crowded housing and social status to infection appears definite.

38. *The Communicable Disease Center.* VERNON B. LINK, Communicable Disease Center, Atlanta, Ga.

The Communicable Disease Center is the combat arm of the Public Health Service in its fight against communicable diseases. The CDC originated on July 1, 1946, when its temporary war-time predecessor, The Office of Malaria Control in War Areas, was converted into a permanent organization. From a concern with malaria primarily, the Center has expanded its interest to include practically all of the communicable diseases with the exception of tuberculosis and the venereal diseases.

During the first nine years of its existence, the Center experienced a decrease in total budget almost every year. This made it appear that those responsible for the appropriation of funds really believed that the communicable diseases had been conquered and that a permanent organization to assist in their control was unnecessary. However, in 1955 two events took place which have had the effect of counteracting this apparent belief: (1) for the first time the CDC received an increase in its total budget; and (2) approval was granted for the construction of a $13,000,000 building to house the headquarters activities of the Center. These two events tend to insure the existence of a Center which has demonstrated its leadership in the control of communicable disease.

A description will be given of the activities now being carried on by the CDC in the various diseases, including plans for the future.

38a. *The Problem of Schistosomiasis in Puerto Rico.* JOSE F. MALDONADO and JOSE OLIVER-GONZALEZ, School of Medicine, San Juan, P. R.

The coprological incidence of *S. mansoni* in six endemic foci has decreased from 22.2 percent in 1953 to 11.3 percent in 1955. The drop was not uniform in an irrigation area, the incidence there tending to increase after a sharp drop in 1954. Soil pollution is still high throughout, no control programs have been instituted and the snail vector is still to be found, although in variable numbers. The improving economic situation, the program for safe water supply to every part of the island, and education, are probably the factors contributing toward the decrease in schistosomiasis. A reassay of the situation should be carried out a few years hence.

2:00 P.M., November 1

SYMPOSIUM

Helminthic Infections as the Cause of Disability and Disease

Moderator, NORMAN R. STOLL

Part I

*39. *Introduction.* (10 mins.) NORMAN R. STOLL, The Rockefeller Institute for Medical Research, New York, N. Y.

*40. *Debilitative Effects and Control of Filariasis.* (15 mins.) JOHN F. KESSEL, School of Medicine, University of California, Los Angeles, Calif. and Institut de Recherches Médicales de l'Océanie Francaise.

*41. *Fasciolopsiasis and Opisthorchiasis as Components of Public Health in Asia.* (15 mins.) ELVIO H. SADUN, Communicable Disease Center, Atlanta, Ga.

*42. *Echinococcus Disease in Northern North America.* (15 mins.) JOHN B. POOLE, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

*43. *Wandering Nematodes as a Cause of Disability and Disease.* (15 mins.) PAUL C. BEAVER, School of Medicine, Tulane University, New Orleans, La.

*44. *Peripatetic Parasitologist.* (15 mins.) HAROLD W. BROWN, School of Public Health and Administrative Medicine, Columbia University, New York, N. Y.

Part II

*45. *Helminth Production in Livestock: Tonnage, Carrying Costs and Control.* (12 mins.) DONALD C. BOUGHTON, E. I. duPont de Nemours and Company, Wilmington, Del.

*46. *The Active Absorption of Certain Metabolites by Helminths.* (12 mins.) JACK W. DAUGHERTY, The Rice Institute, Houston, Tex.

*47. *The Collagenase-like Enzymes of Skin Penetrating Helminths.* (12 mins.) ROBERT M. LEWERT and CHANG-LING LEE, The University of Chicago, Chicago, Ill.

*48. *The Development of a Study on the Axenic Growth* in vitro *of* Nippostrongylus muris *to the Adult Stage.* (12 mins.) PAUL P. WEINSTEIN and MYRNA F. JONES, National Institutes of Health, Bethesda, Md.

* To be published in the JOURNAL.

9:00 A.M., November 2

†49. *A Comparison of the Efficacy of Single Doses of Piperazine Salts for the Treatment of Cases of Ascariasis.* JOSEPH H. MILLER, CLYDE SWARTZWELDER, and ROBERT W. SAPPENFIELD, Louisiana State University School of Medicine and Charity Hospital, New Orleans, La

Sixty patients with ascariasis were treated with single weekly doses of piperazine citrate, piperazine adipate and piperazine phosphate (Antepar). The infection was considered eliminated when *Ascaris* eggs were not demonstrable in formalin-ether concentrations of stools one week after treatment. The dosage in all cases was 70 mg. per pound with a maximum of 3.0 gm. Stoll egg counts before treatment ranged from 400 to 94,300 *Ascaris lumbricoides* eggs per ml.

Thirty patients received piperazine citrate syrup. The infection was eliminated in 83 percent after the initial dose. Four patients required a second dose and one patient a third dose to effect a cure.

Fifteen patients received piperazine adipate suspension. The cure rate after a single dose was 67 percent. Five patients required a second dose to eliminate the infection.

Fifteen patients received piperazine phos-

† Read by title.

phate suspension or chewing wafers. The infection was eliminated in 87 percent after the first dose. Two patients required a second dose.

No practical difference in the efficacy of the three piperazine salts was demonstrated. The syrup was preferable to a suspension or chewing wafer for pediatric use. No significant untoward reactions to the drugs were observed. The authors suggest the following two dose schedule for the treatment of ascariasis with piperazine citrate: an initial single dose of 70 mg. per pound with a maximum of 3.0 gm. repeated one week later. This regime eliminated the infection in 97 percent of the cases treated with piperazine citrate.

†50. *The Use of Piperazine Citrate in the Treatment of Apparent Partial Intestinal Obstruction Due to Ascariasis.* ROBERT W. SAPPENFIELD, CLYDE SWARTZWELDER, and JOSEPH H. MILLER, Louisiana State University School of Medicine and Charity Hospital, New Orleans, La.

Seven cases with symptoms of intestinal obstruction and heavy *Ascaris* infection without other obvious etiology were admitted to Charity Hospital from January 1, 1955 to June 1, 1956. All patients were less than 4½ years of age and ranged in weight from 16 to 34 pounds. Six patients were females.

The patients presented with vomiting and abdominal distention of 3 days duration or less. Other symptoms such as mild diarrhea, fever, and abdominal cramping were present in less than 50 percent of the patients. Hemograms, urinalyses and abdominal x-rays were non-revealing except for distention of the intestine and a leukocytosis in one child.

Supportive therapy included abdominal decompression by Levine tube and suction in three cases, repeated enemas in five cases, and parenteral fluids in four cases.

Piperazine citrate was given orally when possible. When it was necessary to give the medication directly through the Levine tube, suction was discontinued for one hour. Two regimens were followed: 35 mg. per pound *b.i.d.* for 6 doses or 70 mg. per pound in a single dose, repeated once when necessary.

All patients improved dramatically. Surgical intervention was not necessary. Supportive therapy was discontinued by the third hospital day in all cases. Large numbers of worms were passed between the second and the fifth day after initiation of therapy. Six of the seven patients were discharged after only six days of hospitalization. Piperazine citrate may be a valuable addition to the therapy of possible partial intestinal obstruction due to ascariasis.

51. *Investigation of the Action of Piperazine on* Ascaris lumbricoides. STATA NORTON and E. J. DE BEER, The Wellcome Research Laboratories, Tuckahoe, N. Y.

Tension recordings were made of the *in vitro* motility of the pig Ascaris. The effects of drugs on the whole worm and on the eviscerated anterior half of the worm were studied.

Some compounds, such as acetylcholine, did not appear to penetrate the cuticle, since they had no effect on the whole worm, but were markedly effective when applied to the split, eviscerated worm.

The split worm was essentially a nerve-muscle preparation and responded to many pharmacologically active compounds in a fashion similar to vertebrate striated muscle *in vitro* (e.g. the frog rectus abdominis preparation). Compounds depolarizing the vertebrate neuromuscular junction (succinylcholine and decamethonium) caused a contracture of the Ascaris muscle, while d-tubocurarine blocked the response to acetylcholine. Piperazine, which causes a flaccid paralysis when applied over a period of hours to an intact worm (1), rapidly blocked the acetylcholine contracture of the split worm.

Electrical stimulation of the muscle was attempted by placing one electrode in the cavity of the anterior half of worms which were eviscerated but not split. The second electrode was in the bath fluid. In this preparation d-tubocurarine and piperazine did not block the electrically induced muscle spasm. This evidence indicates that piperazine causes paralysis of Ascaris by directly blocking the neuromuscular junction.

(1) Standen, O.D. Brit. Med. J., ii, 21 (July 2, 1955).

52. *Effect of Piperazine Hexahydrate on Succinate Production by* Ascaris lumbricoides. ERNEST BUEDING and GEORGE M. FARROW (Introduced by W. W.

FRYE), Louisiana State University School of Medicine, New Orleans, La.

The presence of high concentrations of succinate in the perienteric fluid of *Ascaris lumbricoides* has been reported recently (E. Bueding and G. M. Farrow: Am. J. Trop. Med. and Hyg., *5*, 382, 1956). In the course of a study concerned with the rate of succinate formation by this parasite it was found that during incubation for 24 hours at 37°C. in a glucose containing buffered salt solution *Ascaris* produced 0.2 to 0.8 millimoles of succinate per 100 gm. (wet weight). In the presence of paralyzing concentrations of piperazine (0.05 per cent and above, initial pH: 8.1) the amount of succinate excreted into the medium was reduced to an extent of over 80 per cent and the succinate concentration of the worms was decreased significantly. If, following incubation with piperazine, the worms were transferred into piperazine-free medium, succinate production was restored to normal indicating that this metabolic change produced by piperazine was reversible. The possible relationship between this biochemical effect of piperazine and its anthelmintic action will be discussed. (Supported by U.S.P.H.S. research grant #E-668).

53. *Studies of the "Malic Enzyme" and Carbon Dioxide Fixation in* Ascaris lumbricoides. HOWARD J. SAZ and JEANETTE A. HUBBARD (Introduced by CLYDE SWARTZWELDER), Louisiana State University School of Medicine, New Orleans, La.

A soluble "malic enzyme" has been isolated from homogenates of *Ascaris* muscle (Saz, Fed. Proc., *15*, 612 (1956)). The enzyme has been partially purified and catalyzes the oxidative decarboxylation of l-malate to pyruvate and carbon dioxide. There appear to be at least three major differences between the *Ascaris* "malic enzyme" and that of mammalian, bacterial and plant tissues. First, the enzyme has never before been found in muscle. Second, whereas all previously described systems have an absolute cofactor specificity for either DPN or TPN, the enzyme obtained from *Ascaris* is active with either coenzyme. Third, in contrast of all previously reported "malic enzymes", purified *Ascaris* preprations did not catalyze the decarboxylation of oxalacetate (between pH 5 to 8; with or without added ATP). The *Ascaris* enzyme also catalyzes the reverse reaction. Radioactive carbon dioxide has been fixed into malate. The importance of carbon dioxide fixation *in vivo* has been investigated. After incubation of intact ascarids with glucose and radioactive carbon dioxide, 91 μM of succinate were isolated. It was determined from the isotope content of the succinate that a minimum of 11 μM of carbon dioxide was incorporated into this compound. Therefore, carbon dioxide fixation plays an important role in *Ascaris* metabolism. (Supported by U. S. Public Health Service grant #E-668).

54. *Hematopoietic Stimulating Property of Ascaris Polysaccharide.* JOSÉ OLIVER-GONZÁLEZ, School of Tropical Medicine, University of Puerto Rico, San Juan, P. R.

A polysaccharide was extracted from *Ascaris lumbricoides* from pig, placing the moist and ground adult worm substance in boiling water for 30 minutes, centrifuging and precipitating the polysaccharide from the supernatant with 95 per cent alcohol. The material was partially purified by redissolving in water and reprecipitating with alcohol. It was then sterilized by autoclaving for 30 minutes at 15 lbs. pressure.

Mice of an A strain weighing from 20 to 30 grams, received 10 milligrams daily, given intraperitoneally for 30 days. The animals were killed beginning on the fifth day of injection and thereon every five days until the end of the 30-day period. The liver, spleen, lymph nodes and bone marrow were fixed in Zencker-formol, and sectioned for histological study.

Study of liver sections revealed evidence of hematopoiesis. The process could be observed beginning on the 8th day, but was more intense in the animals inoculated for 30 days. Hematopoiesis is diffuse, the portal fields are enlarged, showing numerous dense masses of cells. Cells of the granulocytic, erythrocytic and lymphocytic series, as well as megakariocytes are evident. Besides extramedullary hematopoiesis, as observed in the liver, there is hyperactivity of the spleen, lymph nodes and bone marrow.

Since the polysaccharide is of a complex nature, consisting of polysaccharides coming from various tissues of the worm, studies

are under way to determine which substance is responsible for the process.

55. *Some Observations on the Sensitization of Mice to Ascaris Perienteric Fluid Proteins.* KENNETH A. LAURENCE and L. O. NOLF, State University of Iowa, Iowa City, Iowa.

Several methods of sensitizing mice to the perienteric fluid proteins of *Ascaris lumbricoides* were attempted. Animals were divided into five groups: (1) normal animals, which received no sensitizing treatment; (2) animals sensitized by an inhalation technique; (3) animals sensitized by painting the skin for 7 days with the protein solution; (4) animals sensitized by three injections of the proteins suspended in Ramons adjuvant; (5) animals which were passively sensitized by receiving immune rat serum.

Sensitization to the proteins was tested by anaphylaxis. Animals which received the painting treatment and adjuvant induced sensitization failed to shock. Those animals which received internasal and passive sensitization showed typical reactions, with 40–50 percent demonstrating fatal anaphylaxis. Normal animals did not shock. Mice which had recovered from an infection with Ascaris showed as well shocking reactions on receiving an intravenous injection of the body fluid.

Electrophoretic patterns of the perienteric fluid show five protein components. Further studies with fractionation of the fluid proteins in an attempt to demonstrate sensitization with individual fractions are in progress.

56. *Studies on the Serology of Visceral Larva Migrans by the Use of Bentonite Flocculation Test.* ELVIO H. SADUN, LOIS NORMAN, and DOROTHY ALLAIN, Communicable Disease Center, Chamblee, Ga.

Purified acid soluble protein fractions were obtained from adult *Toxocara canis* and used in the bentonite flocculation test. Sera from experimentally infected or artificially immunized rabbits gave positive flocculation reactions. Positive reactions occurred between 7 and 20 days following inoculation and persisted for variable amounts of time. Extensive cross-reactions were observed with sera of rabbits infected with *Ascaris lumbricoides*.

Human sera were also tested with *Toxocara* antigen and only titers higher than 1:5 were considered significant. Out of 21 sera from children suspected of being infected with visceral larva migrans, 5 gave positive results. Conversely, all of 174 human sera from asymptomatic Americans were negative. Out of 105 sera from Korean prisoners of war, two were positive.

Normal sera from 166 laboratory and domestic animals were also tested. Occasional sera from rabbits, horses, pigs, sheep and chickens gave non-specific positive results with *Toxocara* antigen. Sera from over 100 wild animals trapped in south Georgia were also tested. Positive reactions were obtained only in 4 out of 47 raccoons and one out of 8 wildcats.

No obvious cross-reactions with sera from humans or experimental animals infected with or immunized against *Clonorchis, Echinococcus, Trichinella, Schistosoma mansoni, Entamoeba histolytica, Toxoplasma, Salmonella, Leptospira, Tularemia, Brucella, Pasteurella pestis, Streptococcus, Malleomyces mallei, Typhus, Poliomyelitis, Lympho choriomengitis, Vaccinia* and *Psittacosis*, were observed. Three out of 6 serum specimens from humans immunized against rabies were positive.

57. *On the Comparative Distribution of Phosphate Esters in* Trichuris vulpis, Ascaris lumbricoides, *and* Strongyloides ratti. CHARLES A. JONES, CLYDE SWARTZWELDER, and STANLEY H. ABADIE, Veterans Administration Hospital and Louisiana State University School of Medicine, New Orleans, La.

A comparative study of the distribution of some phosphorus compounds in three species of parasites has been made. Filariform larvae of *S. ratti* grown in the feces from infected rats; adult parasitic female *S. ratti* from the intestine of infected rats; *T. vulpis* from the ceca of naturally infected dogs; and *A. lumbricoides* spontaneously passed by human subjects were collected and while still living frozen in liquid nitrogen and stored at −15°C until analysed. Organisms were extracted with trichloracetic acid, fractionated with barium and alcohol and the various compounds assayed by appropriate conventional methods. When

required the manipulations were carried out in the cold room at 0°C.

Intriguing and distinctive differences were found in the distribution of some of the phosphate compounds. The filariform larvae and adult parasitic female *S. ratti* and adult *T. vulpis* contained appreciable quantities of a "labile phosphorus" presumably phosphogen phosphorus, possibly arginine phosphate. No such labile fraction of phosphorus was found in *Ascaris*.

The barium insoluble fraction of the TCA extract of *T. vulpis* contained little phosphorus. Adenine and pentose were present in traces only. This finding virtually rules out the occurrence of adenosine polyphosphates in this fraction, in contrast to the finding of ample quantities of these high energy phosphate compounds in the other species studied.

The TCA extracted residues of these parasites were analysed for glycogen which was found to vary in concentration from the 0.8% found in *T. vulpis* to the 0.015% found in *Ascaris*. Several of the phosphate esters formed during glycolysis were found to be present and varied considerably in amount and in their ratios to one another. Lactic acid was present in each of these parasites. The significance of these variations in composition of these parasites which reside in different locations in the intestines of different hosts is under study at present. (This investigation was supported by Research Grant E-543 from the National Microbiological Institute, of the National Institutes of Health, Public Health Service).

58. *An Overwhelming Infection with* Strongyloides stercoralis. H. W. Brown and V. Perna, College of Physicians and Surgeons, Columbia University, New York, N. Y.

A 58 year old Panamanian who had resided in New York City for 36 years died with an overwhelming infection of *Strongyloides stercoralis*. Large numbers of mature female worms, developing eggs and larvae were present in the mucosa of the stomach, duodenum and jejunum. Migrating larvae were found in the mucosa, wall and subserosa of the small and large bowel, in mesenteric lymph nodes, peripancreatic lymphatics, the liver, walls of the portal vein, lungs, capsule of the thyroid and loose connective tissue adjacent to the parathyroids. In view of the patient's urban residence for 36 years, it is probable that she acquired her infection in Panama and that it was maintained through a hyperinfective cycle.

59. *The Effect of Carbon Monoxide on Homogenates of* Trichinella spiralis *Larvae*. Erwin Goldberg and L. O. Nolf, State University of Iowa, Iowa City, Iowa.

It has been demonstrated that the larvae of *Trichinella spiralis* contain the approximate absorption bands of the cytochromes b, c and a ± a_3. In addition, cell-free homogenates of the larvae contain enzymes which catalyze the oxidation and reduction of cytochrome c (Goldberg, 1956). The present report is concerned with the effect of carbon monoxide on the cytochrome system and on the overall respiration of *T. spiralis* homogenates.

Stannard, McCoy and Latchford (1938) reported that carbon monoxide: oxygen gas mixtures increased considerably the endogenous oxygen consumption of intact larvae. In the present experiments with homogenates of the larvae we observed a 10% increase in the oxygen consumption of a system containing either succinate or ascorbate as a hydrogen donor in an atmosphere of 95% CO, 5% O_2. By spectrophotometric methods it was possible to demonstrate a 30% inhibition of the cytochrome oxidase activity of the larvae by carbon monoxide. This inhibition occurred in the dark and was reversed by light.

These results indicate that the larvae of *T. spiralis* are able to oxidize CO to CO_2, as has been reported for some types of vertebrate tissues. In addition to carbon monoxide serving as a substrate, it also functions in the usual way as an inhibitor of cytochrome oxidase.

60. *Effect of Cortisone Treatment on Immunity to Subsequent Reinfection with* Trichinella *in the Rat*. Edward K. Markell, University of California Medical School, Los Angeles, Calif.

Sixty Wistar rats were infected with equal numbers of *Trichinella* larvae by stomach tube. They were then divided into three lots of twenty animals each; one lot was kept as controls, the second lot was

treated with 3 mgm. daily of Cortisone acetate by subcutaneous injection for a period of 30 days, and the third group was treated with the same dosage of Cortisone for a period of 60 days. The animals were allowed to recover from their initial infection, and approximately 5 months later each group was again divided into two subgroups, one of which was reinfected with *Trichinella* in the same manner as before. Upon autopsy of the entire group of animals six weeks after the second infection, it was found that as measured by counts of larvae in the musculature, the animals not treated with Cortisone had developed a high degree of immunity to reinfection, whereas those treated with Cortisone had developed little or no immunity from their initial infection.

61. *The Use of a Metabolic Antigen in the Serological Diagnosis of Trichinosis in Humans and Hogs.* Lois Norman and Elvio H. Sadun, Communicable Disease Center, Chamblee, Ga.

A total of 1,086 serum specimens from humans suspected of being infected with *Trichinella* were tested by the flocculation reaction with metabolic and somatic antigens. Similar results were obtained in 1,026 specimens. Of the sera giving contrasting results, 52 were positive with the metabolic and 8 were positive with the somatic antigen. Of 50 specimens from individuals with proven trichinosis or with diagnoses highly suggestive of trichinosis, 45 were positive with both antigens, 3 were negative with both antigens and 2 gave contrasting results. Whereas with human sera the metabolic antigen seemed to confer to the flocculation test, a greater sensitivity, no significant difference could be observed in 108 sera from hogs.

Of 163 sera from wild animals, 155 gave negative reactions with both antigens. The fact that negative results were obtained with most of the sera from wild animals in spite of their being infected with various helminths, added to the information obtained from uninfected laboratory-raised hogs and rabbits suggests that both antigens are specific for trichinosis.

Absorption of homologous antibodies from immune sera was attempted. Antibodies were absorbed by homologous antigen in greater proportion than by the heterologous antigen. However, a certain degree of cross-reaction was observed between the metabolic and the somatic antigen. Protein determinations of the two antigens were made and the relative amounts compared.

*62. *Factors Influencing the Appearance of Precipitates on Larvae of* Litomosoides carinii *in Cotton Rat and White Rat Sera.* Norman T. Briggs (Introduced by J. A. Scott), The University of Texas Medical Branch, Galveston, Tex.

It has been previously reported that characteristic oral precipitates are formed on larvae of *Litomosoides carinii* cultured in serum from both cotton rats and white rats which had received infective larvae subcutaneously.

In the experiments reported here precipitate formation has been demonstrated in serum taken from cotton rats 25 days after they had received small numbers of larvae subcutaneously. More marked reactions have been observed in serum from rats which had received three times as many larvae given at one time or in three successive doses at five day intervals. Larvae cultured in serum from white rats treated similarly showed comparable responses.

In other experiments cotton rats were infected and their developing worms recovered 7 days later. Three successive transfers of such worms were made at weekly intervals into the abdominal cavity of a second group of cotton rats. Serum obtained from the latter rats 25 days after the first transfer induced only sporadic and barely discernable precipitates. As a control to this experiment cotton rats were given infective larvae intraperitoneally in numbers equal to the transferred worms. The serum obtained 25 days later consistently induced characteristic precipitates. It is interesting that in spite of this good precipitate formation the control rats yielded relatively few developing worms, mostly from the pleural cavity. On the other hand, a relatively high percentage of the worms were recovered from the rats which received 7 day worms and whose sera showed almost no precipitate formation. Almost all of these worms were in the abdominal cavity.

* Read by title.

63. *The Early Induction and Persistent Effect of Immunity to the Filarial Worms of Cotton Rats.* ETTA MAE MACDONALD and J. ALLEN SCOTT, The University of Texas Medical Branch, Galveston, Tex.

We have previously shown that the filarial worms, *Litomosoides carinii*, 24 days after they are introduced as infective larvae into previously infected cotton rats are significantly shorter and less advanced in development than similar worms in previously uninfected cotton rats. We have also shown that worms transferred surgically at 7 days from previously uninfected rats to previously infected rats do not show retarded growth in comparison with worms similarly transferred to previously uninfected rats. The growth retarding influence is evidently exerted only in the first 7 days. We have proposed the hypothesis that since little growth occurs normally in the first 7 days, the principal effect of the retarding influence is expressed after the first 7 days. The present experiments were designed to test this hypothesis. Cotton rats were infected twice at an interval of one week. About 30 days later they were again infected. After 7 more days the worms from the last infection were transferred to previously uninfected rats which were killed 17 days later. A second group of rats were infected for the first time when the first group received their third infection and the worms transferred as before. A third group received infective larvae for the first time when the second group did but these worms remained in the same animals for the entire 24 days. The worms of the two latter groups did not differ in length or degree of development, but those of the first group were significantly shorter and less developed than those of the second and third groups. These experiments seem to provide adequate support of our hypothesis.

64. *A New Epidemiological Field Kit.* DONALD L. PRICE, Second Army Area Medical Laboratory, Fort George G. Meade, Md. and Walter Reed Army Medical Center, Washington, D. C.

Field work in connection with a project entitled "Filarial Parasites in Mammals" made evident the need for some compact unit for carrying equipment in the field. A field kit, designed by the author and built of plywood was tested for a variety of tasks under field conditions. Detailed modifications were decided upon through field experience and two new kits were constructed of aluminum.

The new units were light and equipped with a carrying strap. Compartment size and arrangement was adapted to standard Army equipment. A removable drawer and test tube rack incorporated as part of the original design were made interchangeable for greater utility. The base of the kit is divided into five equal compartments for stock bottles and one compartment running the full width of the kit for general use. The kit opens by means of a piano hinge into a working surface supported on one side by two folding legs and on the other by the base of the kit. Under the working surface four compartments of equal size are used for general storage.

The kit has proved invaluable for collecting blood or serum, preparing blood smears, collecting arthropod vectors, and dissecting or operating on small mammals in the field.

After a year of use in the field and shipment around the world by various modes of transportation the kits remain in good condition.

64a. *The Treatment of Ascariasis with Piperazine Citrate in Typhoid Fever Patients.* A. PENA-CHAVARRIA, C. LIZANO, and H. XIRINACHS, Hospital San Juan de Dios, San Jose, Costa Rica.

Thirty-six typhoid fever patients harboring infections with *Ascaris lumbricoides* were treated with piperazine citrate, provided by Burroughs, Wellcome and Company. Patients ranged from 4 to 70 years in age. Egg counts on twenty patients ranged from 100 to 43,000 per gram of feces. Dosage ranged from 1.5 to 4.0 grams, usually divided into two doses in one day. Stool specimens of 11 patients were negative after a single day of treatment, the other 9 became negative after a second treatment. No untoward reactions referable to treatment occurred. One patient heavily infected with *Balantidium coli* had negative stools for this parasite after treatment. One patient had intestinal hemorrhages after treatment, apparently due to typhoid ulceration. The flaccid condition of the Ascaris worms passed after this medication appears to minimize the possibility of intestinal perforation by the worms.

2:00 P.M., November 2

†65. *Attempts to Transmit Rabbit Fibromas and Papillomas by Means of a Nematode.* ROBERT C. RENDTORFF and AIMEE WILCOX, National Institutes of Health, Columbia, S. C. and University of Tennessee, College of Medicine, Memphis, Tenn.

66. *Comparison of the Antioxyurid Activity of NF-23 (Ethyl 3-(5-Nitro-2-furyl)-acrylate) and Piperazine Citrate Against the Mouse Pinworm,* Syphacia obvelata. MICHAEL P. NATT, RICHARD H. GUSTAFSON, and JOHN R. O'CONNOR, Eaton Laboratories, Norwich, N. Y.

A number of nitrofuran compounds were tested for antioxyurid activity against a naturally occurring infection of *Syphacia obvelata* in CFW mice. One compound, designated as NF-23 (Ethyl 3-(5-Nitro-2-furyl)acrylate), was found to be particularly active against the mouse pinworm, and in view of its low order of toxicity in mice, rats and dogs, it was given further consideration as a possible oxyuracide. To determine whether slight modifications in the molecular structure of NF-23 had any effect on this activity, a number of analogs were synthesized and evaluated. The results indicated that the vinylog and the alkyl moiety are essential for activity. However, with an increase in the length of the ester portion of the molecule, there was a corresponding reduction in antioxyurid activity. NF-23 was more active than gentian violet, terramycin and piperazine citrate when medicated to mice twice daily for four consecutive days. The antioxyurid activities of NF-23 and piperazine citrate were compared following their administration to mice as a single medication dose, and twice daily for one, two, four and eight days. NF-23 appeared to have greater activity when the medication period was extended beyond the first day. Both compounds were tested against the various developmental stages of the parasite. Piperazine citrate was active against all stages of the parasite with the exception of the very immature worms. Contrastingly, NF-23 did not show preference for any developmental stage of the mouse pinworm.

67. *Chemoprophylaxis of Enterobiasis.* RODNEY C. JUNG, SAMUEL M. EMERSON, and BENNETT SEWELL, Tulane University School of Medicine, New Orleans, La.

The efficacy of piperazine citrate used therapeutically and prophylactically against pinworm was studied in a home for 90 boys aged 4 to 14 years all infected with pinworm. At the beginning of the study six daily cellulose adhesive anal impression tests were made on each child. Those children having four or more positive tests were considered heavily infected. Administration of 5 ml. of syrup of piperazine citrate (1 ml. = 100 mg. piperazine hexahydrate) three times daily for one week failed to cure 5 of 24 heavily infected boys, but the same dosage given for 2 weeks failed to cure only 1 of 25. The same dose of piperazine citrate given in tablet form for one week failed to cure 4 of 23 boys. Tests were positive until the fifth day of treatment in over half the children cured and until the sixth day in one-fourth. Children cured of their infection who received a single weekly dose of 15 or 45 ml. of syrup of piperazine citrate were to some extent protected from reinfection. Eleven of 23 receiving 15 ml. and 12 of 15 receiving 45 ml. failed to become reinfected during an observation period of 20 and 7 weeks respectively. Of 22 controls all became reinfected during the same period. In those individuals who became reinfected in spite of receiving a weekly dose of piperazine the infection was lighter than in the controls. There were no instances of intoxication due to the drug.

‡68. *The Efficacy of Short and Long Regimes for the Treatment of Enterobiasis with Piperazine Citrate.* CLYDE SWARTZWELDER, JOSEPH H. MILLER, ROBERT W. SAPPENFIELD, and FRANCES MARTINEZ, Louisiana State University School of Medicine and Charity Hospital, New Orleans, La.

Three regimes were employed for the treatment of 82 patients for enterobiasis with piperazine citrate syrup (Antepar). The dosage was 30 mg. per pound, with a daily maximum of 2 grams. The treatment was considered effective if pinworm eggs

†Abstract withheld from published group upon request of author(s).

‡Read by title.

were not demonstrable on 7 scotch tape swabs within 2 weeks after completion of therapy.

The first series of patients were treated for two alternate weeks. The total daily dosage was divided and given in two equal doses. Twenty-six out of 27 patients (96 per cent) were treated successfully with this schedule.

In order to reduce the duration of the treatment period and eliminate the interruption of medication, the second group of patients received the anthelmintic for 14 consecutive days. The infection was eliminated in 30 of 33 patients (91 per cent).

The treatment schedule was reduced and simplified further in another series of patients. The drug was administered in a *single daily dose*, after breakfast, for *6 consecutive days*. Twenty-one of 22 patients (95 per cent) were cured with this regime.

These studies indicate that a 6-day course of therapy with a single daily dose of piperazine citrate syrup, at the dosage scale employed, may be equally efficacious in eliminating pinworm infections as 14-day treatment schedules with divided daily doses. The reduction in duration and cost of medication, without loss of therapeutic efficiency, are significant factors in the treatment of this common and frequently familial infection.

69. *Histochemical Assay of the Secretions from the Acetabular Gland Complex of Cercariae of* Schistosoma mansoni. M. A. Stirewalt, Naval Medical Research Institute, Bethesda, Md.

Secretions from the acetabular gland complex were obtained in Kahn tubes and assayed histochemically. That the deposits contain material from both pre- and post-acetabular glands has been established by supravital staining. The deposits were insoluble in organic and inorganic solvents except glacial acetic, concentrated formic, performic, and sulfuric acids. They were resistant to the action of pepsin, but were digested by trypsin. Histochemical assay indicated that the secretion was probably either a muco- or glycoprotein.

70. *Experiments on Anti-Schistosome Circumoval Precipitating Antibody in Mice.* Coit M. Coker and Jose Oliver-Gonzalez, School of Medicine, University of Puerto Rico, San Juan, P. R.

In a series of experiments utilizing the laboratory mouse as a convenient experimental host, information was sought as to time of appearance of circumoval precipitating antibody in *Schistosoma mansoni* infections, longevity of the antibody under conditions of egg-only infections and of passive transfer, and modification of the host response by manipulative techniques. Some mice were given experimental infections, some were given intraperitoneal injections with living schistosome eggs, some were injected intraperitoneally with strongly circumoval positive human serum, and others, treated variously, were given cortisone. The results were measured in terms of the circumoval test, which is indicative although not strictly quantitative. The precipitins first appeared in the sera of infected mice at about 50 days after infection. In egg-injected mice the precipitins were noted by the 46th day, requiring repeated antigenic stimulus, and persisted for at least 112 days after final egg injection. Fragments of eggs, the egg shell in particular, persisted for as long as 112 days in the body cavity environment, where they were encapsulated by a tissue reaction. The presence of passively transferred antibody along with living eggs did not accelerate initial antibody formation. The half-life of circulating circumoval antibody (passively transferred) was not in excess of 6 days. Cortisone treatment of the host did not affect the longevity of circulating antibody but seemed to have a definite suppressive effect upon the initial formation of circumoval antibody.

71. *The Development of Antibodies in Schistosome Infection as Measured by the Miracidial Immobilization Reaction.* Laurence B. Senterfit, The Johns Hopkins University and Hospital, Baltimore, Md.

The sera of animals infected with *Schistosoma mansoni* have been shown to be capable of immobilizing the miracidia of this parasite. The reaction has been shown to be a reproducible reaction that can be titered using serial dilutions of serum.

Comparisons of the time of appearance of the immobilization reaction and other *in vitro* reactions in the serum showed that these reactions all became positive at approximately the same time. In studies on

human sera it was shown that there was a correlation between the immobilization and the cercarial agglutination. No correlation was shown between the immobilization reaction and the circumoval precipitate or the circumcercareal precipitate.

The immobilizing antibody has been stimulated by the injection of antigen-adjuvant emulsions prepared from various stages of the parasite. Each of these antigenic mixtures stimulated immobilizing antibody production. It has been shown that the immobilizing antibody may be absorbing from the serum by the use of dry, powdered adult worms, cercariae, and eggs. From these experiments the conclusion has been made that the immobilization reaction is due to antibody in the serim. It has also been concluded that the antigen that stimulates the production of immobilizing antibody is present in all stages of the parasite found in the mammalian host.

These studies have shown that the immobilizing antibody is at a high level during the acute infection and at a relatively lower level, or completely absent, in chronic infections.

72. *Interstrain Variation of the Egg Shape of* Schistosoma japonicum. H. F. Hsü and S. L. Li Hsü, State University of Iowa, Iowa City, Iowa.

Three strains of *Schistosoma japonicum*—Chinese, Formosan, and Japanese—were used for the present study. For each strain, 300 eggs from each of the following twelve animals—3 dogs, 3 cats, 3 hamsters, and 3 mice—were measured. The length and width of the eggs were recorded and the shape index of each egg was accordingly calculated The significance of differences of the egg shape among different strains and hosts were studied.

For each strain, another 50 eggs from an infected cat were measured daily for 30 consecutive days and the intrahost daily variation of the egg shape was studied. In addition, for each strain, eggs from 30 infected mice were measured for the study of the interhost variation of the egg shape. Fifty eggs were measured from each mouse.

A significant interstrain variation of the egg shape of *Schistosoma japonicum* was found. Each strain showed its own characteristic egg shape. The egg shape of the Formosan nonhuman strain differs not only from that of the Chinese and Japanese human strains but also the egg shape of the latter two differs. These findings confirm our previous assumption that the zoophilic schisotosome in Formosa is not a distinct species which differs from *Schistosoma japonicum*, but a special geographical strain of the same species of the human schistosome which occurs in other parts of Oriental countries. On the other hand, it is of interest to note that there is a morphological basis in differentiation of the different strains of this parasite.

73. *Studies on the Phenol Oxidase of the Liver Fluke,* Fasciola hepatica. Tag E. Mansour (Introduced by Henry E. Meleney), Louisiana State University School of Medicine, New Orleans, La.

Recent investigations have shown that lysergic acid diethylamide, serotonin and other related indolamines exert a marked stimulatory effect on the rhythmical activity of the liver fluke *Fasciola hepatica* (Mansour, Fed. Proc., *15*, 1480, 1956). This stimulatory effect was shown to be peripheral and was mediated by the central ganglia. On the other hand, epinephrine had no effect on muscular activity. In an attempt to explain why epinephrine did not stimulate the parasites it was found that homogenates of the flukes catalyze the oxidation of this compound at a high rate. This oxidation was not due to the action of an amine oxidase because oxidation of the side chain containing the amine group did not occur. Furthermore, homogenates of *Fasciola* catalyzed the oxidation of phenols which have no amine side chain such as catechol. These observations indicated that this oxidation was due to a phenol oxidase. The oxidative product of epinephrine was found to be adrenolutine, a derivative of adrenochrome. Other substrates which were oxidized by this enzyme were tyramine, hydroxytyramine, dihydroxyphenylalanine, norepinephrine and tyrosine ethyl ester. Compounds lacking the phenolic structure such as ephedrine and serotonin were not oxidized. Serotonin and other related indolamines on the other hand inhibited the activity of the phenol oxidase. This inhibition of serotonin was found to be competitive. The activity of this oxidase was completely abolished in the presence of diethyl thiocarbamate

(1×10^{-3}M). This inhibition could be reversed by the addition of copper ions, strongly suggesting that this oxidase is a copper enzyme. (Supported by grants from the U. S. Public Health Service and from Swift and Company).

74. *Further Studies on Experimental Ocular Trichomoniasis.* JULIA T. WELD and B. H. KEAN, Cornell University Medical College, New York, N. Y.

The serial transmission of *Trichomonas vaginalis* in the eyes of rabbits and the pathologic changes in these eyes have already been reported. The affinity of the parasite for lens substance was considered noteworthy.

In the present study attempts were made to follow the fate of the parasite by cultures and by histologic examination of infected eyes at intervals of one minute to 41 days. These experiments indicated that, within one hour after inoculation into the anterior chamber, the parasite "disappeared"—only to reappear in the aqueous humor or in the lens two or three days later. The explanation for this phenomenon is not clear but the possible existence of a tissue phase may be considered. When parasites were injected into the vitreous, a cellular and fibrinous exudate developed and parasites could be identified without difficulty 4 to 11 days after inoculation. In these experiments, the parasites were relatively close to the lens but invasion was not seen. Parasites were present at some distance from the retina.

Preliminary observations on the effect of drugs and elevation of temperature as factors in elimination of the parasites were made.

75. *Growth of* Trypanosoma lewisi *in the Heterologous Mouse Host.* D. R. LINCICOME, Naval Medical Research Institute, Bethesda, Md. and Howard University, Washington, D. C.

It was reported earlier (Lincicome, 1955) that *Trypanosoma lewisi* could be grown in the mouse (heterologous) host by restriction of the mouse's diet and simultaneous administration of normal rat serum.

Under these conditions 2 separately isolated lines of *T. lewisi* have now been grown in mice for more than 100 serial passages. At intervals the trypanosomes have been tested to determine whether they would grow in the absence of either normal rat serum or starvation of the heterologous host. At the 75th passage of one isolate the parasites developed without restriction of the host's diet and have continued to grow through 18 subsequent passages in the mouse although normal rat serum still remains a requisite.

The 3 lines of parasites thus produced are compared with respect to the parasitemia produced, death of the host, interval elapsed to next passage, development and duration of maximum populations in peripheral blood. Although there is considerable variation in the growth of *T. lewisi* under heterologous conditions in the mouse, there does not appear to be any gross metabolic change in the parasite or alteration of its ability to develop normally in its homologous host.

The growth of *T. lewisi* in the mouse host supported by normal rat serum alone, in the absence of any metabolic change due to its presence in an heterologous environment, would therefore seem to offer an opportunity for the identification of the growth-promoting factor(s) present in rat serum.

76. *Antigen Dose-Response Relationship in Immunization of Rats to* Trypanosoma equiperdum. WILLIAM CANTRELL and GORDON D. BETTS, University of Louisville School of Medicine, Louisville, Ky.

Rats infected with *Trypanosoma equiperdum* and cured with oxophenarsine acquired a rapidly developing, highly specific immunity to reinfection. The immunized rats were not completely protected, however, and after a variable period were killed by a relapse strain which differed antigenically from the original population. The relationship between the period of protection and the intensity of parasitemia at the time of immunization (antigen dose) is complex due to the presence of more than one antigenic type of trypanosome in the population living in the rat at any time. As a result the curve relating protection to antigen dose is characterized by a series of plateaus and steep transition zones connecting the plateaus. The transition zones correspond to threshold antigen doses at which the various antigenic types become plentiful enough

to act as effective immunizing stimuli. Whether varying the antigen dose affects protection, therefore, depends on the location of the various antigen doses with respect to transition zones. We have made several experiments in which the antigen dose was varied over a 2-fold to 4-fold range without any effect on the period of protection. On the other hand, when a wider range of antigen doses was used so that one or more transition zones was included, a marked difference in the protection was observed at certain levels of antigen dose. The curves obtained in this way make possible inferences about the number and relative prevalence of different antigen types in a population.

77. *Studies on the Activity of a Bis-quinaldine (PR-445) Against* Trypanosoma gambiense *in Experimental Animals.* G. F. Otto, J. C. Moetsch, and R. U. Schock, Abbott Laboratories, North Chicago, Ill.

A number of the bis-quinaldines have shown remarkable activity against either *Trypanosoma gambiense* or *T. congolense* in mice. As would be expected, no one member of the series has shown the same order of activity against both parasites. In order to learn something of the mode of action, one of these, N,N′-bis-(2-methyl-4 quinolyl)-,6-hexanediamine dihydrochloride (PR-445), has been studied extensively against *T. gambiense* in experimental animals. Caged animals tolerated very large intramuscular injections—mice, 400 mg./kg.; rats, 120 mg./kg.; rabbits, 160 mg./kg.; dogs, 100 mg./kg.; and monkeys, 134 mg./kg. Only the monkeys presented even a slight local swelling under these conditions. This is in sharp contrast to the immediate tissue breakdown and subsequent distal necrosis following the intramuscular injections of much smaller doses of pentamidine.

Mice infected intraperitoneally with 25,000,000 *T. gambiense* invariably die within two to three days when not treated. When treated, 24 hours after infection with PR-445, there was 100% survival following 15 mg./kg.; 30 to 80% following 6.0 to 10.0 mg./kg. and even some survivals following as little as 2.0 mg./kg. Surviving animals were reinfected (10,000,000 parasites) at 28-day intervals as long as they survived. Under these conditions following 15 mg./kg. the average survival time for PR-445 was 64 days, for suramin and pentamidine each 33 days; at 40 mg./kg. the average survival times were 90 days for PR-445, 63 days for suramin and 51 days for antryeide, and 33 days for pentamidine. In both rabbits and rats also PR-445 provided longer chemical prophylaxis than did either suramin or pentamidine. For instance, when the first infection was given to rats three months after a single intramuscular injection of 50 mg./kg., 4 of the 5 receiving PR-445 were completely protected whereas 5 of 5 receiving pentamidine and 5 of 5 receiving suramin became infected. Thus, on a dose for dose basis PR-445 has consistently offered longer protection than has either pentamidine or suramin.

*78. *Effects of Nitrofurazone Therapy of Chronic Cases of African Sleeping Sickness Refractory to Previous Treatment with Various Known Trypanocides.* F. Evens and A. Packchanian, Institute for Tropical Medicine "Princess Astrid," Leopoldville, Belgian Congo and School of Medicine, The University of Texas, Galveston, Tex.

79. *The Electrophoretic Pattern of the Serum Proteins in Experimental Hemoflagellate Infections.* Harry Seneca and James Sang, College of Physicians and Surgeons, Columbia University, The Presbyterian Hospital, and Beth El Hospital, New York, N. Y.

1. In hamsters, Leishmania donovani causes a slight depression of the total serum proteins, a reversal of A/G ratio, a marked depression of albumin and gamma globulin, a marked increase in alpha globulin and hyperlipemia. Trypanosoma gambiense causes a marked depression of the total proteins, albumin and gamma globulin are depressed and alpha$_1$ globulin is increased. Trypanosoma rhodesiense causes depression of the total proteins and albumin and an increase of alpha$_1$ and alpha$_2$ globulins. Trypanosoma cruzi was not pathogenic to the hamsters.

2. T. rhodesiense in guinea pigs, rats and

* Abstract withheld from published group upon request of author(s).

mice did not show marked changes in the electrophoretic pattern of the serum proteins. Albumin and B globulin were depressed in the guinea pigs. Alpha and gamma globulins were increased in rats. In mice, the total proteins were slightly depressed but B globulin was increased.

3. Trypanosoma cruzi in d b a mice showed slight depression of the serum proteins, otherwise the pattern was normal.

7:00 P.M., November 2

Dinner Session

Presentation of the Walter Reed Medal by N. PAUL HUDSON, Chairman of the Award Committee

† Presidential Address—"The Interrelations of Nutrition and Infectious Diseases in the Tropics," ASA C. CHANDLER, Rice Institute, Houston, Texas

9:30 A.M., November 3

80. *A Modified Shaffer-Frye Technique for the Cultivation of* Entamoeba histolytica. RICHARD E. REEVES, HENRY E. MELENEY, and WILLIAM W. FRYE, Louisiana State University School of Medicine New Orleans, La.

The Shaffer-Frye technique for cultivation of *E. histolytica* in the presence of a penicillin-inhibited monobacterial associate has been successfully employed in this laboratory. Four strains of amoebae (200 E, HK-9, F-22 and DKB) were carried with routine 24 or 48 hour transfers for a period of approximately one year. During this time parallel experiments led to some progress in the direction of simplifying the manipulations required by this technique, particularly by omitting the vaseline overlay and eliminating the necessity for preconditioning the medium with bacterial growth.

Our routine cultures have now been changed from the original Shaffer-Frye technique as follows. The medium base consists of Trypticase, glucose, sodium chloride, $K_2HPO_4 \cdot CH_2O$, thiomalic acid, methylene blue (optional) and sodium hydroxide to pH 7.0 ± 0.1. The "streptobacillus" is grown on a similar medium to which yeast extract has been added. The medium base is autoclaved in 12 ml. amounts in 15 x 125 mm. screw cap culture tubes.

When the amoeba transfer is made the medium is completed by adding to each tube an amount of streptobacillus culture, or washed bacterial cells, containing approximately 0.1 to 0.15 mg. of bacterial nitrogen (Kjeldahl), 0.25 ml. of sterile, unfiltered horse serum and 5000 units of penicillin in saline. Inoculated with five to ten thousand amoebae and incubated at 37° in a slanted position, these cultures regularly produce 100,000 to 500,000 amoebae within 48 to 72 hours. The growth and multiplication appears to be as good as with the original Shaffer-Frye technique.

81. *Bacteria-Free Cultures of* Entamoeba histolytica *with Chick Embryo Extract.* RICHARD E. REEVES, HENRY E. MELENEY, and WILLIAM W. FRYE, Louisiana State University School of Medicine, New Orleans, La.

Entamoeba histolytica cultures maintained on a modified Shaffer-Frye medium were transferred to culture tubes containing the same base medium with penicillin and serum, but without added streptobacilli. In place of the bacteria 2.0 to 2.5 ml. of a sterile centrifuged chick embryo extract was employed. After the second transfer in the chick embryo medium it was no longer possible to subculture the streptobacillus with penicillinase under optimum conditions for anaerobic bacterial growth. Portions of subsequent cultures were treated with active penicillinase after which they were plated and subcultured in tubes under conditions favorable for either aerobic or anaerobic bacterial growth, with negative results.

The growth-promoting activity of the embryo extract appeared to be associated

† To be published in the JOURNAL.

with a particulate fraction which remained in suspension when centrifuged for 20 minutes at 700 to 1000 times gravity, but which sedimented rapidly at 2400 times gravity. Freezing and thawing the active extract diminished, but did not entirely destroy the ability of the extract to support amoeba growth. The activity was entirely lost, however, by heating the extract to 70°.

Amoebae survived for a week or more in the embryo extract tubes. Our routine transfers of these cultures were made twice weekly. The serial transfers with the unfrozen extract showed about four-fold multiplication, with the frozen extract seldom more than two-fold.

Four strains of amoebae (DKB, 200 E, F-22 and HK-9) have been obtained in bacteria-free cultures by this technique. At the time of this writing (June, 1956) only the 200 strain has been maintained for as many as ten transfers before accidental aerobic contamination of the embryo extract terminated the series.

82. *The Effect of Penicillin G on Two Strains of Small Race* Entamoeba histolytica *in Culture.* James G. Shaffer, Phyllis Conner, and Gladys Rosenthal, *The* Chicago Medical School, Chicago, Ill.

In repeated unsuccessful attempts to transfer two strains of small race *Entamoeba histolytica* from mixed bacterial flora on diphasic medium to the Shaffer-Frye medium, it was observed that the penicillin G routinely used in S-F medium seemed to produce certain interesting morphological changes in the amebas. Further investigation showed that when penicillin G was added to these same strains of *E. histolytica* on the diphasic media, similar changes were observed. Stained preparations from these cultures revealed many forms whose morphology had a striking resemblance to *Blastocystis hominis.*

Several possibilities for the origin of these Blastocystis-like structures have been considered and investigated, but all the evidence now at hand indicates that these forms are being derived directly from the trophozoites of these small race *E. histolytica* strains. The implications of these observations are of great interest.

83. *Histological Observations on the Influence of a Special Diet Used in Experimental Amebiasis in Guinea Pigs.* John E. Lynch and Vincent J. Dardin, Pfizer Therapeutic Institute, Maywood, N. J. and Georgetown Medical Center, Washington, D. C.

Numerous references appear in the literature demonstrating the importance of nutrition in resistance to the development of infectious diseases. This is especially true in the case of parasitic infections of the intestinal tract. Changes in the intestinal environment of a nutritional nature may severely alter the relationship between the host and the parasite.

For the past three years, the senior author has been evaluating compounds for antiamebic activity in guinea pigs. In order to obtain a high order of infection, it has been necessary to employ a special diet described by Roine and Elvehjem. Such a procedure has been accepted as a normal prerequisite for the development of acute ulcerative amebiasis in the guinea pig.

The present report is the result of our studies to determine what influence the special diet has on the development of experimental amebiasis in guinea pigs.

It has been stated that a symbiotic relationship between the ameba and one or more species of bacteria is necessary before amebic invasion of the tissues occurs. Using a commercial pellet ration, attempts were made to implant selected bacterial species prior to inoculation with *Endamoeba histolytica.* Since such a procedure did not bring about any higher degree of infection than could be produced by merely inoculating *E. histolytica* intracecally, it occurred to one of us (JEL) that perhaps a histological study of the ceca of the guinea pigs maintained over varying periods of time on the special diet might demonstrate some variation in the cellular pattern of the cecum. If this were the case, such information might offer an explanation of the development of acute ulcerative amebic infection, at least in guinea pigs, and might interest other investigators to conduct similar studies in other animals with other disease processes, which appear to be influenced by nutritional environment.

Data are presented to show the results of our histological studies which revealed al-

teration in the structure of the ceca of guinea pigs maintained on the special diet.

*84. *Studies on the Ameba-Bacteria Relationship in Amebiasis. II. Some Concepts on the Etiology of the Disease.* BRUCE P. PHILLIPS and PATRICIA A. WOLFE, National Institutes of Health, Bethesda, Md. and Lobund Institute, University of Notre Dame, South Bend, Ind.

85. *Progressive Blinding Central Serous Choroidosis Associated with Amebiasis Responding to Antiamebic Therapy.* HENRY E. HAMILTON and A. E. BRALEY, University Hospitals and College of Medicine, State University of Iowa, Iowa City, Iowa.

Central choroiditis is commonly a progressive disease failing to respond specifically to any therapy. Eight patients are presented who have had a macular lesion characterized by a cystic area associated with hemorrhage into the choroid and retina. The vitreus was slightly detached over these lesions and there were floaters in this area. The pigment epithelium was hyperplastic around these cystic areas. The patients also had findings compatible with active amebiasis and were free of evidence of tuberculosis, brucellosis, diabetes mellitus, or other active disease. These patients were treated only with antiamebic drugs such as diodoquin, chloroquine and carbarsone. The response to treatment was prompt with healing of the macular lesions, improvement or complete restoration of vision and the disappearance of the clinical signs and symptoms of amebiasis.

Patients with other macular lesions or retinal toxoplasmosis were treated similarly but without change in the progressive course of the disease.

Photographs of the retinal lesions associated with "amebic central serous choroidosis" and those due to vascular involvement, diabetic retinopathy, toxoplasmosis and other macular lesions will be presented for purpose of differential diagnosis.

The central choroidosis associated with amebiasis is thought to represent hypersensitivity but evidence of direct tissue invasion by the parasite has not been excluded.

Because of the apparent reversibility of this particular lesion it is important for the ophthalmologist, physician, and parasitologist to study all patients with pigmented cystic lesions in the macula for evidence of amebiasis.

86. *Clinical and Electrophoretic Studies of Serum Proteins in Human Amebiasis.* EDWARD O. COMER and CHARLES A. JONES (Introduced by CLYDE SWARTZWELDER), Veterans Administration Hospital and Tulane University School of Medicine, New Orleans, La.

Since no studies of changes of serum gamma globulin levels in human subjects infected with *Entamoeba histolytica* have been reported heretofore, a clinical and electrophoretic investigation was carried out. Fifty-two patients were included in this survey. A liver biopsy was also performed on 28 of these patients. Four patients had acute amebic dysentery. The mean value as determined by electrophoresis for serum gamma globulin in these subjects was 0.66 grams per cent before therapy. Following treatment this level was 0.81 grams per cent. Total proteins and albumin fractions were low during the entire course of observation. Forty-eight patients had chronic amebiasis. In these the gamma globulin fraction was 0.93 grams per cent, albumin 2.55 grams per cent, and total proteins 6.01 grams per cent before treatment. Fourteen patients were available for follow-up studies by electrophoresis. The value for these fractions did not fluctuate significantly from those obtained before therapy. It appears that patients with acute amebic dysentery may have low gamma globulin concentrations during infection, and these appear to return to the normal range following eradication of amebic infection. Patients with chronic amebiasis appear to have normal serum gamma globulin levels before and after treatment. Liver biopsy failed to substantiate demonstrable hepatic lesions existing with colonic amebiasis in those patients who had no definite clinical signs of amebic hepatic involvement.

87. *Attempted Control of Amebiasis in an Institution by Mass Therapy.* M. M. BROOKE, RALPH H. HEEREN, GRACE M. SAWYER, and DOROTHEA STONER, Communicable Disease Center, Atlanta, Ga., Iowa State Department of Health, Des

* Abstract withheld from published group upon request of author(s).

Moines, Iowa, and Woodward State Hospital and School, Woodward, Iowa.

In an institution of approximately 1600 mentally deficient patients, examination of single stool specimens from a sample of 730 individuals indicated that at least 29 per cent harbored *Entamoeba histolytica*. Prevalence rates from building to building ranged from 7 to 65 per cent. Preliminary tests of mass therapy in the building with the highest prevalence showed that single courses of oxytetracycline did not eradicate all infections, and that the incidence of infection rose to one half of the pretreatment level by the end of six months.

Later, all of the patients received therapy on two occasions, six months apart. On the first occasion, the patients were divided into four approximately equal groups and each group received one of the following four courses of therapy: vioform, carbarsone, oxytetracycline and a combination of oxytetracycline and carbarsone. On the second occasion the same medications were administered, but the group assignment was changed for each drug.

The prevalence of *E. histolytica* in the institution was reduced by mass therapy, but the infection was apparently eradicated in only three of the eight buildings. The effectiveness of therapy in the various buildings seemed to be related to the prevalence rates prior to medication and to the general level of personal hygiene of the patients. Although the control program was not designed specifically to evaluate the various drugs, the relative effectiveness of the four courses of therapy is discussed.

88. *Residual Hepatic Dysfunction After Recurrent Amebiasis.* HAMILTON H. ANDERSON, TIEN BOH KWEE, and THOMAS L. NELSON (with the assistance of RUTH MCELROY and VIRGINIA TISDALE), Sonoma State Hospital, Eldridge, Calif. and University of California School of Medicine, San Francisco, Calif.

During the survey of a ward of mentally defective children, it was noted that 17 of 105 patients exhibited hepatomegaly. A variety of liver function tests indicated that 14 of these patients had hepatic impairment. Nine revealed *E. histolytica* in their stools, and 7 others had a recent history of recurrent, drug-refractive amebiasis.

Thirty patients, among those examined, were found to harbor *E. histolytica*. These were given a new dipiperonyl derivative (MA-307) together with 11 others having hepatomegaly but not presently harboring amoebae in their intestinal contents.

Of the patients thus chosen and isolated for experimental therapy, approximately half had reduction in liver size within 2 weeks of therapy. An equivalent number had reversal of impaired function tests. The remainder are under observation for a 3 month follow-up period.

89. *The Nature of Amebic Hepatitis.* B. H. KEAN, Cornell University Medical College, New York, N. Y.

Although the relationship between intestinal amebiasis and abscess of the liver has been known for almost one hundred years, the concept of amebic hepatitis has been developed only in the last two decades. The syndrome as described consists of fever, hepatomegaly, hepatic tenderness, leukocytosis, laboratory evidence of hepatic dysfunction in a patient with a history of intestinal amebiasis, and disappearance of the symptoms following anti-amebic therapy. Analysis of 5,000 routine autopsies in Panama, 200 fatal cases of amebiasis from the Armed Forces Institutes of Pathology, and histologic examination of 75 liver biopsies in patients with the clinical syndrome of amebic hepatitis failed to reveal satisfactory histologic evidence of diffuse amebic hepatitis. Current study of the liver in 200 cases of ulcerative colitis suggests that the syndrome of amebic hepatitis is not specific but is due to the presence of ulceration of the intestinal tract, and that the liver abnormalities disappear when the ulceration of the intestinal tract is eliminated.

SCIENTIFIC EXHIBITS

The following exhibits were displayed in the Green Room of the Jung Hotel.

"The Tropical Fire Ant, a Tropical Agent of Disease Introduced into the United States." R. C. JUNG and V. J. DERBES, Tulane University, School of Medicine, New Orleans, La.

"Review of Studies on the Treatment of Human Helminthiases with Piperazine Salts."

CLYDE SWARTZWELDER, JOSEPH H. MILLER and ROBERT W. SAPPENFIELD, Louisiana State University School of Medicine and Charity Hospital, New Orleans, La.

"Acute Respiratory Illness of Adenovirus (RI-APC) Etiology in Military Populations." M. R. HILLEMAN, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

"The Status of Yellow Fever in Middle America." The Pan American Sanitary Bureau, Regional Office of the World Health Organization, Washington, D. C.

"Development of Insect Repellents." E. J. DEHNE, Army Environmental Health Laboratory, Army Chemical Center, Md.

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Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

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Tables should be typed on separate sheets and be serially numbered.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

Snyder, T., and Meleney, H., 1941. The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21:** 63–73.

Citation in the text should be by author and date.

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All papers should end with a brief summary.

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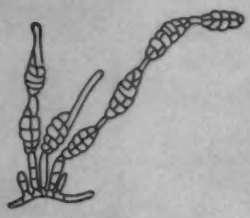
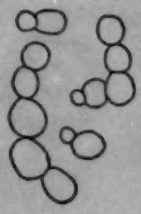
MYCOLOGY



DIAGNOSTIC

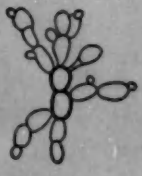
. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:



Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:



Bacto-Sabouraud Maltose Broth
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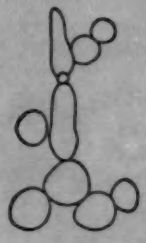

Bacto-Yeast Morphology Agar
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Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
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